Microglial regulation of satiety and cognition

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Abstract

Microglia have been known for decades as key immune cells that shape the central nervous system (CNS) during development and respond to brain pathogens and injury in adult life. Recent findings now suggest that these cells also play a highly complex role in several other CNS functions. In this review, we will provide a brief overview of established microglial functions in development and disease. We will also discuss emerging research suggesting microglia are important in both cognitive function and the regulation of food intake. With respect to cognitive function, current data suggest microglia are not indispensable for neurogenesis, synaptogenesis or cognition in the healthy young adult, but crucially modulate and support these functions. In doing so they are likely important in supporting the balance between apoptosis and survival of newborn neurons and in orchestrating appropriate synaptic remodelling in response to a learning stimulus. We also explore the possibility of a role for microglia in feeding and satiety. Microglia have been implicated in both appetite suppression with sickness and obesity and in promoting feeding under some conditions and we discuss these findings here, highlighting the contribution of these cells to healthy brain function.

Key words: cognition, microglia, neuroinflammation, satiety

1. Introduction

Microglia are resident innate immune cells of the central nervous system (CNS). They are in direct contact with and interact with neurons and synapses, as well as other glial cells in the CNS parenchyma [1-3]. During brain development, they are important in regulating neuronal survival and death, synaptogenesis and angiogenesis and in adults they are key in responding to brain injury and pathogens [4]. Recently, additional roles for these cells in the healthy brain are starting to emerge along with evidence that subtypes with distinct functions are important in central responses to challenge [5-8]. In this review, we will present a brief overview of microglia's known roles in development and in responding to pathogens and injury, but will also discuss new evidence suggesting microglia play an integral role in at least two fairly disparate physiological functions in the healthy adult animal: cognition and satiety signalling. We will confine our discussion here to microglia and direct the reader to excellent recent reviews covering the roles of astrocytes and other CNS cell types [9, 10]. Understanding of these novel roles for microglia is being made possible because of the development of several new pharmacological and transgenic strategies for manipulating these cells. Traditional pharmacological strategies including liposomal clodronate [11] and minocycline [12, 13] have recently been added to with colony stimulating factor receptor 1 (CSF-R1) kinase inhibitors PLX3397 [14, 15] and PLX5562 [16, 17] (so far exclusively used in mice). In the transgenic space, there are now total knockouts for several microglia-specific genes that results in death of these cells (e.g. chemokine, CX3C motif, receptor 1 (-/-) [cx3cr1(-/-)], complement receptor 3 [CR3(-/-)], and DAP12(-/-)[18]). The diphtheria toxin receptor (DTR) model $CX3CR1^{CreER}$:iDTR in mice exploits the idea that at least a population of microglia are longer-lived than circulating monocytes [19]. *Cx3cr1-Dtr* rats offer a strategy for rat microglia-specific ablation [20]. Each of these strategies carries limitations; ones of specificity with the traditional pharmacological agents, developmental in the total knockouts. The PLX

compounds require a protracted time frame for microglial depletion [21], precluding their use in neonatal or acute applications. There is also a suggestion that only a subset of microglial genes may be affected [22]. The CX3CR1^{CreER}:iDTR mouse may be limited by at least some of the microglia rapidly replacing themselves [23]. In the *Cx3cr1-Dtr* rat, monocytes are also affected [20]. Nonetheless, together these strategies provide highly useful insight into novel microglial roles.

2. Microglia

2.1. A brief history of microglia

Microglia were first identified in 1899 by Franz Nissl and W. Ford Robertson in their description of "reactive glial elements" [24]. The term microglia was coined by Pio del Rio-Hortega in the early 20th century, who described their activation in response to brain injury; their non-neuronal elements that were distinct from the neuroectodermal macroglia, oligodendroglia and astroglia; and their origin from the mesoderm [24-26].

Despite some early controversy [27-29], it is now established that microglia likely have a yolk sac origin, deriving from undifferentiated hematopoietic precursors and moving into the brain in two waves, initially via interstitial migration prior to the development of the cerebral blood vessels and the blood-brain barrier, as well as before the production of monocytes in hematopoietic tissue [30, 31], and later through the blood circulation [30]. By approximately P42 in rodents [23, 32], microglial numbers have refined to those maintained throughout adulthood and at this stage microglia make up approximately 5-15% of cells within the brain [33]. Historically thought to be remarkably long-lived based on H^3 thymidine labelling [34], recent data now suggest that at least a proportion of microglia turn over much more rapidly, with a possible replacement rate of 96 days for the whole population in the rodent given a uniform turnover

[23]. In this study, turnover rate was estimated using 5-bromo-2'-deoxyuridine (BrdU) incorporation - a thymidine analogue that is incorporated into the S phase of the cell cycle during DNA replication [23]. In an unrelated study using samples from two human patients, microglia were found to have an average age of 4.2 years and to renew at a rate of 28% of the microglia in the cortex being replaced per year; calculated using comparison of the ^{14}C levels in the microglial DNA relative to the atmospheric ^{14}C curve, with cells as old as the individual having ^{14}C levels corresponding to the level at birth and newer cells having ^{14}C levels corresponding to the number of divisions made [35].

Through development, microglia express an age-dependent gene signature and morphology, shifting from an ameboid morphology (large round soma, few to no processes) in early life to a ramified morphology (smaller soma, many complex processes) as the animal matures [36, 37]. In the adult animal, microglial morphology differs throughout the brain depending upon the tissue type, region, age, sex and presence or absence of an immune challenge or injury [5, 24]. Very broadly, however, microglia display a highly ramified "surveillant" morphology under unstimulated conditions, at which time they are highly motile, with continuous extension and retraction of their thin long processes, while the soma remains relatively stationary. In the presence of a significant injury, however, microglial cells rapidly extend their processes toward the stimulus and take on a more ameboid morphology, as seen with two-photon microscopy [38, 39].

2.2. Microglia's role in brain development

During embryonic and postnatal brain development, microglia play essential roles in several processes of brain network refinement [4]. They are aligned along the axonal tracts, supporting their development [18]. They refine neuronal networks by promoting programmed cell death

and phagocytosis of apoptotic cells [40]. They also regulate synapse formation and pruning, through the removal of synaptic spines [41, 42]. Their key importance in these processes is supported by many studies, not least a recent case of a human baby born without functional microglia due to a CSF-1R mutation. In this case, brain networks were severely disrupted, with increased neuron number and aberrant neuronal connectivity [43].

Neuronal apoptosis and survival

During embryonic development, high densities of microglial cells are seen alongside specific developing axonal tracts, particularly on dopaminergic axons [18]. Elimination of microglia, using cell-depletion approaches and cx3cr1(-/-), CR3(-/-), and DAP12(-/-) mutants, causes an increased dorsal outgrowth of these axons, while maternal immune activation (and thus microglial activation*)* restricts dorsal extension and is associated with a pronounced ventral increase in dopaminergic innervation [18].

During the late stages of cortical neurogenesis, microglia are ameboid in morphology, phagocytic in activity, and actively phagocytose neural precursor cells (NPC) [11]. If microglial activation is suppressed at this time, or if microglia are depleted, there is a significant increase in the number of NPCs as a consequence of a reduction in phagocytosis [11]. Conversely, during early postnatal development microglia promote the survival of cortical neurons in the subcortical white matter in an insulin-like growth factor-1-dependent manner, as determined using minocycline as well as CD11b-DTR and *Cx3cr1*-deficient mice [44].

Synaptic pruning and formation

During early brain development, microglia also play a key role in eliminating supernumerary synapses and refining synaptic networks [3]. For example, during rodent development, the

retinal ganglion cell inputs from both eyes overlap until the beginning of the second postnatal week and are thereafter rearranged to eye-specific regions. This process involves selective pruning of these projections and is known to be mediated by the activation of the complement pathway with microglial cells pruning those synapses that localize complement cascade initializing protein q (C1q) and complement 3 (C3) [42, 45]. Deficiencies in this pathway can be encouraged either by eliminating the CR3 receptor (with CR3 knockout mice) found on microglia or by reducing the neuronal ligands C1q or C3 (with knockout mice), leading to reduced synaptic pruning. Such impairment of synaptic elimination induces retinogeniculate projection segregation deficits that can be observed long-term in the adult brain [46].

Pruning of supernumerary synaptic elements has also been shown in the hippocampus during the first postnatal weeks of development in rodents via electron and stimulated emission depletion microscopy of excitatory post-synaptic density (PSD-95) and pre-synaptic terminals (SNAP25) in the cytoplasm of microglial cells, suggesting that microglial cells phagocytose the synaptic elements [41]. Paolicelli *et al.* have also demonstrated that pruning of supernumerary dendritic spines is delayed in the hippocampus of Cx3cr1 knockout mice, in which there is a delay of microglial cell recruitment [41]. These mice also show maturation deficits in the functional properties of their excitatory synapses [41, 47]. Microglia have also been identified to play a role in synaptic pruning in the cerebral cortex in non-human primates as is indicated by the similarities in the time course of synaptic and microglial gene pathway changes [48, 49]. Recent evidence suggests that a direct phagocytic role in development may not be the major mechanism by which microglia regulate synaptic maturation, rather that they may act via a process of trogocytosis, or “nibbling” of synaptic elements to reshape the synaptic material [50].

Alongside, microglia's role in synapse elimination, it is now clear that these cells also influence early life synaptic formation and functional synapse maturation, likely through the release of growth factors and cytokines [51, 52]. In particular, microglial elimination in juvenile mice impairs the turnover of spines in the pyramidal neurons of the motor cortex resulting in fewer dendritic spines in this region [19]. This reduction of dendritic spine formation was reproduced with specific knockout of brain-derived neurotrophic factor (BDNF) in microglia, suggesting that microglial production of this growth factor is essential for the formation of new spines in the neonatal cortex and this influences performance during motor learning and fear conditioning in adulthood [19]. Particular cytokines are also known to impact synaptic functions and could therefore be considered as important microglia-dependent mediators of postnatal synaptic maturation. For instance, interleukin (IL)-1β is a proinflammatory cytokine produced by microglia as well as other CNS cells. Deficits in hippocampal synaptic plasticity and in cognitive functions programmed by microglial maturational delay in Cx3cr1 knockout mice are rescued by antagonising IL-1β signalling [53].

2.3. Microglia's role in responding to pathogens and injury

Early on in the scientific history of microglia, it was established that these cells are highly responsive to pathogens and injury in both the developmental phase and in adulthood [24-26]. In response to acute injury, microglia phagocytose cellular debris, which can be seen in bulbous formations at their ends with *in vivo* two-photon live imaging [38, 39]. This function is mediated typically by two types of receptors that respond to the "eat me" "don't eat me" signals (phagocytic machinery and signalling pathways) upon recognizing pathogen- and danger-associated molecular patterns on pathogens, from metabolic products or dying/dead cells [24, 54]. Microglial functional diversity is demonstrated in their differential responses to specific stimuli. When stimulated by microbial components, such as lipopolysaccharide (LPS),

microglial cells react by activating a phagocytosis program that involves activation of toll-like receptors (TLRs) leading to transcription of pro-inflammatory mediators including cytokines [55]. These cytokines have been found to be neurotoxic at high concentrations and to also increase inflammation (e.g. tumor necrosis factor -α; TNF-α), and induce fever and other aspects of sickness behaviour (e.g. IL-6) [56, 57]. Cytokines also regulate presentation of cell surface molecules involved in microglial activation and microglial-neuronal interactions (e.g. interferon γ; IFNγ) [24]. Importantly, humans, unlike rodents, have relatively low microglial TLR4 expression and no detectable IFNγ receptor expression. These differences need to be taken into consideration when translating rodent data to humans [58, 59]. Alternative to this pro-inflammatory response, when microglial cells are challenged by apoptotic cells or myelin debris, they respond by releasing anti-inflammatory agents that downregulate the effects of pro-inflammatory cytokines and promote neuronal survival [60]. Cytokines such as IL-10, IL-4 and transforming growth factor (TGF)-β are anti-inflammatory and modify cell surface receptor expression on microglia and also downregulate the expression or attenuate the effects of pro-inflammatory IL-1β and TNF-α [61].

Clearly microglia are important for perinatal brain development and disruptions to their early activity can have a lasting impact on cognitive function, at least. Microglia's role in the presence of pathogens and injury is also relatively well understood [38, 39]. However, the function of these cells while in "surveillance" mode in the adult brain is only just being recognised. Evidence now suggests these dynamic cells may have an ongoing role in synaptic remodelling, cognition, and other functions, as the animal progresses through adulthood.

3. Microglia's role in cognition

3.1. Microglia in cognition

Microglia are well-known to be involved in the cognitive dysfunction associated with a variety of diseases, as well as with ageing. Several excellent reviews detail our current knowledge on this topic with respect to pathological conditions [62-64] and so we will focus here principally on how these cells may be integral in cognition in the healthy young adult. Recent advances in microglial transgenic models and other methods for manipulating microglial cells have enabled us to identify that these cells may not be indispensable for adult neurogenesis, synaptogenesis, and cognition to occur but, rather, may modulate and support these functions. However, the direction of their contribution remains surprisingly unclear.

There is some evidence to suggest microglial activation is detrimental to cognition and that removing microglia altogether may be beneficial. Cope and colleagues have recently shown that 10 weeks of high-fat diet in the mouse leads to microglial activation in the hippocampus, as well as cognitive deficits, and preventing this activation with knockdown of the fractalkine receptor or with minocycline restores cognition [65]. Although microglial activation outside of the hypothalamus is rarely seen with short-term high-fat feeding, there is evidence to suggest that it can occur with longer exposures and that this may be linked with cognitive dysfunction [66-68]. In this case, microglia may be encouraged in excessive pruning of dendritic spines by an over-activation of the complement signalling pathway initiated at the level of the neuron, since annexin-V, a compound that blocks microglial phagocytosis by binding to "eat me" phosphotidylserine residues on dying cells, also completely restores cognition [65]. In ageing rats, microgliosis and microglial priming are also associated with cognitive decline and microglial priming in the amygdala, in particular, is linked to poor cognitive outcomes [69-72]. On the other hand, activation of microglia in young animals in a spatial learning task is associated with successful performance of that task [73] and is in accordance with a possible

role for microglia in supporting survival of neurons at the appropriate stage of maturity to respond to the task.

If microglial activation is universally detrimental to cognitive function, one might expect that cognition would be improved in the absence of these cells and impaired as they repopulate. Although there is evidence that this might occur under some conditions, these findings are not ubiquitous. Elmore and colleagues have shown that long-term near-complete microglial ablation in mice with a CSF-1R inhibitor does not negatively impact learning and memory, and may benefit spatial memory in the Barnes maze [15]. In our own studies we have seen that acute microglial ablation in our *Cx3cr1-Dtr* transgenic rat that allows diphtheria toxin (DT) to specifically ablate microglia and monocytes (which express the fractalkine receptor, Cx3cr1), does not impair novel object recognition or place recognition memory (De Luca, Sominsky, Spencer et al, 2019, unpublished observations). Likewise, acute pharmacological suppression of microglial activity does not affect cognition in non-obese mice [65]. Notably, Elmore and colleagues have shown in aged rats [74], and we have demonstrated in the young (De Luca, Sominsky, Spencer et al, 2019, unpublished observations), that repopulating microglia (after treatment with CSF-R1 inhibitor, PLX5622 in the former study and after ablation with DT in *Cx3cr1-Dtr* transgenic rats in the latter) transiently support an improvement in cognitive function. On the other hand, other work has shown detrimental effects of early microglial repopulation on motor learning in a rotarod task [19] and in social behaviour in a Crawley's sociability task [14].

The differences between these findings may be due to duration of depopulation and degree of repopulation. For instance, Torres and colleagues showed that a short period of microglial ablation, with CSF-R1 inhibitor PLX3397 for 7 days, leads to transient spatial memory

impairments in the Barnes maze with longer escape latencies during training. However, these impairments dissipate with a longer depletion period of 21 days [14], while a longer ablation protocol (2 months of PLX3397) enhances spatial memory in the Barnes maze, with shorter escape latencies and reductions in the average escape latencies across all training days [15]. Timing with respect to repopulation may also be important. For instance, our data with the *Cx3cr1-Dtr* rat suggest there is a transient improvement in memory after microglial ablation that coincides with a time at which the microglia have repopulated in number but display an ameboid "activated" morphology (De Luca, Sominsky, Spencer et al, 2019, unpublished observations). Interestingly, Rivest and others provide data to suggest that neuroprotective bone marrow-derived microglia are recruited to the brain under certain pathological conditions and these infiltrating microglia behave differently from the resident ones. Potentially microglial repopulation in models of ablation involve these peripherally-derived recruited microglia [75, 76]. However, the question remains, how might microglia both support and suppress cognitive function?

3.2. Mechanisms for microglial modification of cognitive function

Under normal physiological conditions, neurogenesis has been found in the subgranular zone (SGZ) of the dentate gyrus [77, 78] and the subventricular zone of the lateral ventricles [79] and is a key substrate for cognitive flexibility and long-term memory. Hippocampal neurogenesis is maintained by the proliferation of neural stem cells located in the SGZ [80, 81]. Only a few newborn cells are incorporated into the circuitry - the majority are presumed to die at the immature neuron stage in the first one to four days of their life [82]. Sierra and colleagues have shown that unchallenged ramified microglia are an essential component of the neurogenic SGZ niche and that microglia physically intermingle with NPCs, neuroblasts and newborn neurons, phagocytosing newborn neurons that are apoptotic [83]. Which neurons survive seems

to depend upon the timing of their birth with respect to their recruitment by learning tasks. Thus, when a stimulus is received, new neurons that are not sufficiently mature to propagate action potentials upon stimulation may be recruited to an apoptotic pathway, while young neurons that are sufficiently mature to respond are further strengthened and are more likely to survive long-term [84-86]. In support of this, undergoing a learning task, such as the radial arm maze, leads to fewer immature neurons (doublecortin (DCX)-positive) surviving in the hippocampus at the conclusion of the task [73]. Likewise, those with the highest learning-induced reduction in newly proliferating cells, as labelled with BrdU, perform the best in the Morris water maze (MWM) [84]. Preventing apoptosis in the late, consolidation, phase of the MWM task, by inhibiting caspase-3, delays acquisition of the spatial memory [85]. It is noteworthy that microglia are highly activated in spatial learning tasks, potentially encouraging or cleaning up after this additional apoptosis [73].

Complicating the story somewhat is evidence to suggest that increased adult neurogenesis in the hippocampus is associated with rewarding experiences such as environmental enrichment, mating or physical activity, all of which have been shown to be positive for cognitive function [87]. Alternatively, aversive experiences commonly associated with microglial activation and inflammation such as stress, immune challenges, aging and social defeat exposure lead to a short-term decrease in the production of new neurons [88]. Interestingly, central inflammation can be associated with a "rebound" increase in neurogenesis above pre-challenge levels once the inflammation has resolved [89]. Together these data suggest adequate neurogenesis is necessary for appropriate cognition, but activity-dependent selection of which of these cells survive is also essential and microglia may be important in regulating this.

Synaptogenesis and synaptic remodelling are also an important mechanism for successful cognition with deficits in synaptic plasticity leading to cognitive disorders [90]. Microglia's role in synaptic plasticity is illustrated by recent research showing synaptic proteins are selectively eliminated by microglia during sleep [91] and cathepsin S, a microglial-specific lysosomal cysteine, is secreted by microglia during the dark (active) phase to decrease the spine density of cortical neurons leading to a reduction in synaptic strength during the following light (inactive) phase [92]. Notably, then, the detrimental effects of microglial ablation on motor learning are linked to a reduction in synapse formation in the motor cortex in the learning phases of this task [19]. Likewise, the detrimental effects of high-fat diet on novel object recognition performance can be restored by preventing excessive microglia-mediated pruning of dendritic spines [65]. These data again suggest that both the absence of microglia and their excessive activity can encourage excess synaptic pruning with a negative outcome for cognition. In this regard, it is clear that a balance of appropriately dynamic dendritic spines is important for learning and memory. For example, females show fluctuations in dendritic spine density by up to 30% across the ovarian cycle but can display equal success in memory tasks while employing different recall strategies [93, 94]. These data suggest that a balance of appropriately active microglia is important for optimal learning and memory and that neither too much nor too little input from these cells is of benefit, that the balance between mature and immature neurons and dendritic spines are key encoders for successful memories and that microglial cells are important in achieving this (Fig. 1).

4. Microglia's role in satiety

There is a strong body of data to support a role for microglia in cognitive function under pathological conditions and so this recent work revealing a more subtle contribution of these

cells in cognition in healthy adults comes as no real surprise. However, the role of microglia in feeding and satiety is a much more recent addition to our knowledge of their repertoire.

4.1. Microglia in satiety in sickness and obesity

Microglia do have an established role in the anorexia associated with sickness behaviour, the collective fever, withdrawal, and appetite suppression that is seen in response to neuroimmune challenge [95]. Microglia are likely to be involved in all aspects of this response, and microglial activation with visfatin or TLR2 activation leads to increases in pro-opiomelanocortin (POMC) neuronal activity and a suppression of food intake co-incident with other aspects of the sickness response [96, 97].

Microglia may also be involved in the dysregulated appetite associated with obesity. Microglial activation is a key factor in obesity and can be seen in the brain prior to evidence of peripheral inflammation [98]. Indeed, high-fat diet feeding leads to early microgliosis that is protective against the detrimental effects of the diet on the health of POMC neurons [99, 100]. Prolonged high-fat diet, on the other hand, is linked with a second wave of microgliosis that occurs in conjunction with POMC cell death [100]. Notably, the degree of microglial activation is well-correlated with the degree of weight gain in mice fed high-fat diet [100, 101] and inhibiting microglial proliferation with Ara-C reduces high-fat diet-induced weight gain [102]. However, it is still unclear if microglial activation is a cause or an attempt-at-a-protective consequence of obesity. Preventing microglial activation with minocycline fails to restore body weight in high-fat diet-fed mice of the same strain as that used for the Ara-C experiments [65]. Likewise, microglial priming associated with ageing does not automatically lead to obesity in this population and circadian rhythm disruption with dim light at night leads to weight gain in the absence of changes in microglial morphology [103]. Notable sex

differences in susceptibility to high-fat diet illustrate that microglial activation may be a compensatory response to the loss of a CX3CL1-CX3CR1 interaction in males since female mice, in contrast, retain normal CX3CL1-CX3CR1 signalling capacity with high-fat diet and are also protected, relative to males, from diet-induced obesity and microgliosis [104].

4.2. Microglia in satiety

Microglia's role in satiety in healthy adults (if any) is also still unclear. Early conflicting evidence has suggested microglia can a) not affect b) suppress and c) promote appetite and feeding. We will discuss this contrasting evidence here.

Evidence that microglia do not affect feeding

Several recent studies have employed the PLX compounds PLX3397, 5622 and 647 to suppress expression of CSFR1 in mice. Whether these compounds ablate microglia [15] or suppress a subset of microglial-related genes [22], these cells can no longer be seen in the brain after 3 weeks' treatment with this compound and microglia-typical functions, such as the response to LPS, are impaired [15]. Microglial-related pathologies can also be improved with these compounds [74, 105]. However, appetite and feeding remain largely unaffected, at least in adults. As such, the few studies that report food intake or body weight after exposure to PLX compounds report them to be unchanged in chow-fed young adult mice [21, 106, 107]. Similarly, studies employing other pharmacological agents to block microglial proliferation, activity, or interactions with neurons (e.g. AraC, mice, 15 μg/μL icv at 0.11 μL/L for 4 weeks [102]; minocycline: mice, 40 mg/kg in drinking water for 2 weeks [65]; annexin-V: mice, 200 μg/kg iv for 3 days [65]) also often show no effect on weight in control animals [65, 102]. It is noteworthy, though, that all these compounds take several days to influence microglia and there are few reports of body weight data early after commencement of drug administration when

microglial activity is beginning to be impaired. In this respect, it is worth observing that Djogo and colleagues found no significant differences between controls and PLX3397-treated mice at 7 days after commencement of treatment, but there was a notable trend towards a reduced body weight at day seven (97 versus 101% of starting weight) and no reported weight measurements earlier than this [21]. Particularly with agents such as the PLX compounds that are administered in the diet, careful early weight measurements and reports of diet consumption will be necessary to clarify if the microglial inhibition alters satiety, the diet is merely unpalatable, or it has no effect at all.

Evidence that microglial activity suppresses feeding

Findings from the above studies notwithstanding, there is also robust evidence that microglia can suppress feeding. Evidence from slice preparations suggests acute microglial activation leads to an inhibition of orexigenic agouti-related peptide (AgRP) neurons and an excitation of anorexigenic POMC neuron firing in the hypothalamic arcuate nucleus (ARC), consistent with microglia's role in sickness behaviour and with microglial activity suppressing food intake [108]. Likewise, inhibiting microglia with icv minocycline acutely increases food intake [108] and caloric restriction suppresses microglial activation [109]. These findings suggest microglia are at least capable of suppressing feeding when activated and may maintain normal feeding drive under unstimulated conditions.

There is also some work to suggest that microglia may be involved in developmental programming of feeding circuitry to register satiety. Specific depletion of microglial leptin receptors with an ObRloxP-Cx3cr1-Cre mouse closely replicates the db/db phenotype of leptin resistance. These mice have hyperphagia and increased body weight. They also have reduced POMC neuron numbers in the ARC and a reduced density of α-melanocyte-

stimulating hormone (α-MSH) projections from the ARC to the paraventricular nucleus of the hypothalamus (PVN) [110]. Likewise, mice with microglial ablation with the CSF-R1 inhibitor, PLX5622, throughout the embryonic period have reduced POMC neuron numbers postnatally along with accelerated weight gain [111]. Although, it must be considered that this manipulation was associated with a number of other abnormalities.

Evidence that microglial activity promotes feeding

As discussed above for models of obesity, there is also some evidence that microglial activation can promote feeding. In humans, suppression of microglial activity with minocycline can lead to weight loss [112], a finding that is opposite to this compound's effects when given icv in the mouse, potentially reflecting its action on the gut when given orally [113]. However, in a mouse model, specific inactivation of microglia with PLX5622 reduces food intake in high-fat diet fed mice (but, as discussed, not in chow-fed [106]). In addition, we have recently demonstrated that microglial depopulation in the *Cx3cr1-Dtr* transgenic rat leads to an acute suppression of food intake and a corresponding weight loss that is restored as the microglia repopulate [20]. This anorexia in the absence of microglia occurs without any evidence of central or peripheral inflammation as assessed by circulating and brain cytokine levels and gene expression; and without other sickness behaviours such as reduced exploratory activity or pica to suggest it is related to an inflammatory response [20].

Our findings in this study [20] suggest that the changes in feeding behaviour are not due to changes in peripheral hormonal signals such as ghrelin and leptin. They are also unlikely to be due to a direct effect on neuronal networks at the level of the ARC, since microglial ablation leads to increased density of neuropeptide Y (NPY) and reduced numbers of neurons expressing POMC, both of which would normally lead to increased food intake. We

speculate that the absence of microglia may lead to a suppression of feeding by enhancing the anorexigenic output of the paraventricular thalamus (PVT) to the basolateral amygdala, a circuit that interacts with the PVT's orexigenic networks to establish valence in satiety-sensing [114, 115]. In support of this idea, we see greater neuronal activation in the PVT in response to feeding in the absence of microglia. Further work will need to directly test the contribution of microglia to the activation of this feeding circuit.

Integrating microglia's role in feeding

Together, these data suggest microglia can influence feeding under certain adverse conditions, such as with obesity and neuroimmune challenge. In these latter cases, microglial activation is associated with anorexia, but it is still not clear if such activation is effecting the anorexia or is a compensatory response to resolve it. There also seems to be a role for these cells in acutely influencing feeding in healthy adults in the absence of neuroimmune or other stimulation. As such, microglia in surveillance mode may help to integrate satiety information at the level of the PVT and other regions to stimulate or suppress feeding [20]. Further evidence of microglia's important role in modulating feeding and satiety (potentially in both directions) lies in a recent study demonstrating these cells alter their substrate utilisation depending upon time of day. This circadian microglial metabolism is disrupted under conditions of high-fat diet [116]. These data raise the possibility of an indirect role for microglia in satiety; that disruptions to these cells may increase or decrease feeding at the wrong times of day with respect to their usual activity and metabolism. Another possibility that remains to be considered is that the role of microglia in satiety differs depending upon brain region or even upon as-yet-unidentified microglial subtypes.

Mechanisms for how microglia respond to and pass on feeding-related cues are yet untested but microglia do retain the capacity to respond directly to free fatty acids via their TLRs [117], and to phagocytose neuronal elements depending upon neuronal signals [11, 38-40], potentially including those derived from feeding-related circuits such as NPY/AgRP and POMC neurons. With respect to circadian rhythm variation, microglia also express a suite of clock genes that are highly responsive to dietary factors [116].

5. Conclusion

Research to date is scratching the surface of what microglia may do in the brain. As our tools improve, so will our understanding of the contribution of these cells to healthy brain function, including day-to-day functions such as feeding, cognition, and others. The data discussed in this review amply demonstrate that the contribution of these immune cells to higher order functions is far from simply explained. We anticipate future research will prove a bi-directional relationship between microglia and both cognition and satiety. In doing this, it will be important to remember that the current knowledge on the diverse roles of microglia is based primarily on our discoveries in the rodent brain, more specifically the mouse brain. Although the transcriptomes of mouse and human microglia are largely conserved, there may also be distinct differences in a number of transcription factors and the expression of genes regulated by these factors [118], but also see [119]. It is therefore essential to continue to expand our understanding of the translational relevance of our mechanistic insights. The field awaits a true integration of neuronal function with microglia and, indeed, other brain cell types to allow a full understanding of how we sense and interact with our environment.

Figures

Figure 1.

Microglial regulation of cognition in the adult brain. Changes in microglial activity alter cognitive function: microglial priming in response to aging or high-fat diet impairs cognition, while microglial activation, as occurs in response to a learning stimulus, leads to optimal cognition; suppression of microglial activity is unlikely to affect it. Microglial regulation of cognitive function may be achieved by their influences on neuronal turnover and synaptic remodelling: in response to a learning stimulus, microglial activity is likely to affect the survival of new neurons in the subgranular zone of the dentate gyrus leading to preferential survival of the more mature new neurons. Learning-induced microglial activation may also orchestrate strategic remodelling of dendritic spines, essential for successful cognition. Figure illustration assistance by Alison Schroeer.

Figure 2.

Microglial regulation of satiety. Evidence suggests changes in microglia can either encourage or discourage feeding and weight gain. Colony stimulating factor 1 receptor: CSF-R1; High-fat diet: HDF; intracerebroventricular: icv; lipopolysaccharide: LPS; toll-like receptor 2: TLR2. Figure illustration assistance by Alison Schroeer.

References

1. Kettenmann H, Kirchhoff F, Verkhratsky A. Microglia: New roles for the synaptic stripper. *Neuron*. 2013;77:10-18
2. Prinz M, Priller J. Microglia and brain macrophages in the molecular age: From origin to neuropsychiatric disease. *Nat Rev Neurosci*. 2014;15:300-312
3. Tremblay ME, Lowery RL, Majewska AK. Microglial interactions with synapses are modulated by visual experience. *PLoS Biol*. 2010;8:e1000527

4. Sominsky L, De Luca S, Spencer SJ. Microglia: Key players in neurodevelopment and neuronal plasticity. *The international journal of biochemistry & cell biology*. 2018;94:56-60

5. Masuda T, Sankowski R, Staszewski O, Bottcher C, Amann L, Sagar, et al. Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature*. 2019;566:388-392

6. Weber MD, McKim DB, Niraula A, Witcher KG, Yin W, Sobol CG, et al. The influence of microglial elimination and repopulation on stress sensitization induced by repeated social defeat. *Biological psychiatry*. 2019;85:667-678

7. Bisht K, Sharma KP, Lecours C, Sanchez MG, El Hajj H, Milior G, et al. Dark microglia: A new phenotype predominantly associated with pathological states. *Glia*. 2016;64:826-839

8. Grace PM, Strand KA, Galer EL, Urban DJ, Wang X, Baratta MV, et al. Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal nlrp3 inflammasome activation. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;113:E3441-3450

9. Kastanenka KV, Moreno-Bote R, De Pitta M, Perea G, Eraso-Pichot A, Masgrau R, et al. A roadmap to integrate astrocytes into systems neuroscience. *Glia*. 2020;68:5-26

10. Santello M, Toni N, Volterra A. Astrocyte function from information processing to cognition and cognitive impairment. *Nature neuroscience*. 2019;22:154-166

11. Cunningham CL, Martinez-Cerdeno V, Noctor SC. Microglia regulate the number of neural precursor cells in the developing cerebral cortex. *J Neurosci*. 2013;33:4216-4233

12. Soch A, Sominsky L, De Luca SN, Spencer SJ. Obesity after neonatal overfeeding is independent of hypothalamic microgliosis. *Journal of neuroendocrinology*. 2019;31:e12757

13. De Luca SN, Ziko I, Dhuna K, Sominsky L, Tolcos M, Stokes L, et al. Neonatal overfeeding by small-litter rearing sensitises hippocampal microglial responses to immune challenge: Reversal with neonatal repeated injections of saline or minocycline. *Journal of Neuroendocrinology* 2017;29:e12540-n/a

14. Torres L, Danver J, Ji K, Miyauchi JT, Chen D, Anderson ME, et al. Dynamic microglial modulation of spatial learning and social behavior. *Brain, Behavior, and Immunity*. 2016;55:6-16

15. Elmore MR, Najafi AR, Koike MA, Dagher NN, Spangenberg EE, Rice RA, et al. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron*. 2014;82:380-397

16. Reshef R, Kudryavitskaya E, Shani-Narkiss H, Isaacson B, Rimmerman N, Mizrahi A, et al. The role of microglia and their cx3cr1 signaling in adult neurogenesis in the olfactory bulb. *Elife*. 2017;6

17. Valdearcos M, Douglass JD, Robblee MM, Dorfman MD, Stifler DR, Bennett ML, et al. Microglial inflammatory signaling orchestrates the hypothalamic immune response to dietary excess and mediates obesity susceptibility. *Cell metabolism*. 2017;26:185-197.e183

18. Squarzoni P, Oller G, Hoeffel G, Pont-Lezica L, Rostaing P, Low D, et al. Microglia modulate wiring of the embryonic forebrain. *Cell Rep*. 2014;8:1271-1279

19. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR, 3rd, Lafaille JJ, et al. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell*. 2013;155:1596-1609

20. De Luca SN, Sominsky L, Soch A, Wang H, Ziko I, Rank MM, et al. Conditional microglial depletion in rats leads to reversible anorexia and weight loss by disrupting gustatory circuitry. *Brain Behav Immun*. 2019;77:77-91

21. Djogo T, Robins SC, Schneider S, Kryzskaya D, Liu X, Mingay A, et al. Adult ng2-glia are required for median eminence-mediated leptin sensing and body weight control. *Cell metabolism*. 2016;23:797-810

22. Srivastava PK, van Eyll J, Godard P, Mazzuferi M, Delahaye-Duriez A, Steenwinckel JV, et al. A systems-level framework for drug discovery identifies csf1r as an anti-epileptic drug target. *Nat Commun*. 2018;9:3561

23. Askew K, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P, et al. Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. *Cell Rep*. 2017;18:391-405

24. Arcuri C, Mecca C, Bianchi R, Giambanco I, Donato R. The pathophysiological role of microglia in dynamic surveillance, phagocytosis and structural remodeling of the developing cns. *Front Mol Neurosci*. 2017;10:191

25. Del Rio-Hortega P. El‘ ‘tercer elemento’’delos centros nerviosus. *Bol. Soc. Esp. Biol.* 1919;9:69-120

26. Del Rio-Hortega P. ‘‘Microglia,’’ in cytology and cellular pathology of the nervous system. *W. Penfield*. 1932:482-534

27. Lewis PD. The fate of the subependymal cell in the adult rat brain, with a note on the origin of microglia. *Brain : a journal of neurology*. 1968;91:721-736

28. Mori S, Leblond CP. Identification of microglia in light and electron microscopy. *The Journal of comparative neurology*. 1969;135:57-80

29. Baron M, Gallego A. The relation of the microglia with the pericytes in the cat cerebral cortex. *Z Zellforsch Mikrosk Anat*. 1972;128:42-57

30. Alliot F, Godin I, Pessac B. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain research. Developmental brain research*. 1999;117:145-152

31. Ashwell K. The distribution of microglia and cell death in the fetal rat forebrain. *Brain research. Developmental brain research*. 1991;58:1-12

32. Nikodemova M, Kimyon RS, De I, Small AL, Collier LS, Watters JJ. Microglial numbers attain adult levels after undergoing a rapid decrease in cell number in the third postnatal week. *Journal of neuroimmunology*. 2015;278:280-288

33. Pelvig DP, Pakkenberg H, Stark AK, Pakkenberg B. Neocortical glial cell numbers in human brains. *Neurobiology of aging*. 2008;29:1754-1762

34. Lawson LJ, Perry VH, Gordon S. Turnover of resident microglia in the normal adult mouse brain. *Neuroscience*. 1992;48:405-415

35. Reu P, Khosravi A, Bernard S, Mold JE, Salehpour M, Alkass K, et al. The lifespan and turnover of microglia in the human brain. *Cell Rep*. 2017;20:779-784

36. Matcovitch-Natan O, Winter DR, Giladi A, Vargas Aguilar S, Spinrad A, Sarrazin S, et al. Microglia development follows a stepwise program to regulate brain homeostasis. *Science*. 2016;353:aad8670

37. Hanamsagar R, Alter MD, Block CS, Sullivan H, Bolton JL, Bilbo SD. Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. *Glia*. 2017;65:1504-1520

38. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. Atp mediates rapid microglial response to local brain injury in vivo. *Nature neuroscience*. 2005;8:752-758

39. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science*. 2005;308:1314-1318

40. Wakselman S, Bechade C, Roumier A, Bernard D, Triller A, Bessis A. Developmental neuronal death in hippocampus requires the microglial cd11b integrin and dap12 immunoreceptor. *J Neurosci*. 2008;28:8138-8143

41. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, et al. Synaptic pruning by microglia is necessary for normal brain development. *Science*. 2011;333:1456-1458

42. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, et al. The classical complement cascade mediates cns synapse elimination. *Cell*. 2007;131:1164-1178

43. Oosterhof N, Chang IJ, Karimiani EG, Kuil LE, Jensen DM, Daza R, et al. Homozygous mutations in csf1r cause a pediatric-onset leukoencephalopathy and can result in congenital absence of microglia. *Am J Hum Genet*. 2019;104:936-947

44. Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, et al. Layer v cortical neurons require microglial support for survival during postnatal development. *Nature neuroscience*. 2013;16:543-551

45. Stephan AH, Barres BA, Stevens B. The complement system: An unexpected role in synaptic pruning during development and disease. *Annual review of neuroscience*. 2012;35:369-389

46. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*. 2012;74:691-705

47. Zhan Y, Paolicelli RC, Sforazzini F, Weinhard L, Bolasco G, Pagani F. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nature neuroscience*. 2014;17

48. Sasaki T, Oga T, Nakagaki K, Sakai K, Sumida K, Hoshino K, et al. Developmental expression profiles of axon guidance signaling and the immune system in the marmoset cortex: Potential molecular mechanisms of pruning of dendritic spines during primate synapse formation in late infancy and prepuberty (i). *Biochemical and biophysical research communications*. 2014;444:302-306

49. Sasaki T, Oga T, Nakagaki K, Sakai K, Sumida K, Hoshino K, et al. Developmental genetic profiles of glutamate receptor system, neuromodulator system, protector of normal tissue and mitochondria, and reelin in marmoset cortex: Potential molecular mechanisms of pruning phase of spines in primate synaptic formation process during the end of infancy and prepuberty (ii). *Biochemical and biophysical research communications*. 2014;444:307-310

50. Weinhard L, di Bartolomei G, Bolasco G, Machado P, Schieber NL, Neniskyte U, et al. Microglia remodel synapses by presynaptic trogocytosis and spine head filopodia induction. *Nat Commun*. 2018;9:1228

51. Bechade C, Cantaut-Belarif Y, Bessis A. Microglial control of neuronal activity. *Frontiers in cellular neuroscience*. 2013;7:32

52. Salter MW, Beggs S. Sublime microglia: Expanding roles for the guardians of the cns. *Cell*. 2014;158:15-24

53. Rogers JT, Morganti JM, Bachstetter AD, Hudson CE, Peters MM, Grimmig BA, et al. Cx3cr1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. *Journal of Neuroscience*. 2011;31:16241-16250

54. Brown GC, Neher JJ. Microglial phagocytosis of live neurons. *Nature Reviews Neuroscience*. 2014;15:209-216

55. Collin M, McGovern N, Haniffa M. Human dendritic cell subsets. *Immunology*. 2013;140:22-30

56. Viviani B, Boraso M, Marchetti N, Marinovich M. Perspectives on neuroinflammation and excitotoxicity: A neurotoxic conspiracy? *Neurotoxicology*. 2014;43:10-20

57. Dantzer R. Cytokine, sickness behavior, and depression. *Immunol Allergy Clin North Am*. 2009;29:247-264

58. Hashioka S, Klegeris A, Schwab C, Yu S, McGeer PL. Differential expression of interferon-gamma receptor on human glial cells in vivo and in vitro. *J Neuroimmunol*. 2010;225:91-99

59. Vaure C, Liu Y. A comparative review of toll-like receptor 4 expression and functionality in different animal species. *Front Immunol*. 2014;5:316

60. Hanisch UK, Kettenmann H. Microglia: Active sensor and versatile effector cells in the normal and pathologic brain. *Nature neuroscience*. 2007;10:1387-1394

61. Sawada M, Suzumura A, Hosoya H, Marunouchi T, Nagatsu T. Interleukin-10 inhibits both production of cytokines and expression of cytokine receptors in microglia. *Journal of neurochemistry*. 1999;72:1466-1471

62. Berger T. Immunological processes related to cognitive impairment in ms. *Acta Neurol Scand*. 2016;134 Suppl 200:34-38

63. Gabande-Rodriguez E, Keane L, Capasso M. Microglial phagocytosis in aging and alzheimer's disease. *J Neurosci Res*. 2019

64. Fourrier C, Singhal G, Baune BT. Neuroinflammation and cognition across psychiatric conditions. *CNS Spectr*. 2019;24:4-15

65. Cope EC, LaMarca EA, Monari PK, Olson LB, Martinez S, Zych AD, et al. Microglia play an active role in obesity-associated cognitive decline. *J Neurosci*. 2018

66. Pepping JK, Freeman LR, Gupta S, Keller JN, Bruce-Keller AJ. Nox2 deficiency attenuates markers of adiposopathy and brain injury induced by high-fat diet. *Am J Physiol Endocrinol Metab*. 2013;304:E392-404

67. Jeon BT, Jeong EA, Shin HJ, Lee Y, Lee DH, Kim HJ, et al. Resveratrol attenuates obesity-associated peripheral and central inflammation and improves memory deficit in mice fed a high-fat diet. *Diabetes*. 2012;61:1444-1454

68. Miller AA, Spencer SJ. Obesity and neuroinflammation: A pathway to cognitive impairment. *Brain Behav Immun*. 2014;42:10-21

69. Barrientos RM, Frank MG, Crysdale NY, Chapman TR, Ahrendsen JT, Day HE, et al. Little exercise, big effects: Reversing aging and infection-induced memory deficits, and underlying processes. *J Neurosci*. 2011;31:11578-11586

70. Barrientos RM, Frank MG, Watkins LR, Maier SF. Memory impairments in healthy aging: Role of aging-induced microglial sensitization. *Aging Dis*. 2010;1:212-231

71. Barrientos RM, Frank MG, Watkins LR, Maier SF. Aging-related changes in neuroimmune-endocrine function: Implications for hippocampal-dependent cognition. *Hormones and behavior*. 2012;62:219-227

72. Spencer SJ, Basri B, Sominsky L, Soch A, Ayala MT, Reineck P, et al. High-fat diet worsens the impact of aging on microglial function and morphology in a region-specific manner. *Neurobiology of aging*. 2018;74:121-134

73. De Luca SN, Ziko I, Sominsky L, Nguyen JC, Dinan T, Miller AA, et al. Early life overfeeding impairs spatial memory performance by reducing microglial sensitivity to learning. *Journal of neuroinflammation*. 2016;13:112

74. Elmore MRP, Hohsfield LA, Kramar EA, Soreq L, Lee RJ, Pham ST, et al. Replacement of microglia in the aged brain reverses cognitive, synaptic, and neuronal deficits in mice. *Aging cell*. 2018;17:e12832

75. Simard AR, Rivest S. Neuroprotective properties of the innate immune system and bone marrow stem cells in alzheimer's disease. *Mol Psychiatry*. 2006;11:327-335

76. Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, et al. Microglia in the adult brain arise from ly-6chiccr2+ monocytes only under defined host conditions. *Nature neuroscience*. 2007;10:1544-1553

77. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, et al. Neurogenesis in the adult human hippocampus. *Nature medicine*. 1998;4:1313-1317

78. Henze DA, Wittner L, Buzsaki G. Single granule cells reliably discharge targets in the hippocampal ca3 network in vivo. *Nature neuroscience*. 2002;5:790-795

79. Curtis MA, Kam M, Nannmark U, Anderson MF, Axell MZ, Wikkelso C, et al. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science*. 2007;315:1243-1249

80. Kempermann G, Jessberger S, Steiner B, Kronenberg G. Milestones of neuronal development in the adult hippocampus. *Trends in neurosciences*. 2004;27:447-452

81. Bonaguidi MA, Wheeler MA, Shapiro JS, Stadel RP, Sun GJ, Ming GL, et al. In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. *Cell*. 2011;145:1142-1155

82. Ma DK, Kim WR, Ming GL, Song H. Activity-dependent extrinsic regulation of adult olfactory bulb and hippocampal neurogenesis. *Annals of the New York Academy of Sciences*. 2009;1170:664-673

83. Sierra A, Encinas JM, Deudero JJ, Chancey JH, Enikolopov G, Overstreet-Wadiche LS, et al. Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell*. 2010;7:483-495

84. Dobrossy MD, Drapeau E, Aurousseau C, Le Moal M, Piazza PV, Abrous DN. Differential effects of learning on neurogenesis: Learning increases or decreases the number of newly born cells depending on their birth date. *Mol Psychiatry*. 2003;8:974-982

85. Dupret D, Fabre A, Dobrossy MD, Panatier A, Rodriguez JJ, Lamarque S, et al. Spatial learning depends on both the addition and removal of new hippocampal neurons. *PLoS Biol*. 2007;5:e214

86. Mouret A, Gheusi G, Gabellec MM, de Chaumont F, Olivo-Marin JC, Lledo PM. Learning and survival of newly generated neurons: When time matters. *Journal of Neuroscience*. 2008;28:11511-11516

87. Leuner B, Gould E. Structural plasticity and hippocampal function. *Annual review of neuroscience*. 2010;61:111-140, c111-113

88. Ming GL, Song H. Adult neurogenesis in the mammalian central nervous system. *Annual review of neuroscience*. 2005;28:223-250

89. Chamaa F, Bitar L, Darwish B, Saade NE, Abou-Kheir W. Intracerebroventricular injections of endotoxin (et) reduces hippocampal neurogenesis. *J Neuroimmunol*. 2018;315:58-67

90. Ramiro-Cortes Y, Hobbiss AF, Israely I. Synaptic competition in structural plasticity and cognitive function. *Philos Trans R Soc Lond B Biol Sci*. 2014;369:20130157

91. Choudhury ME, Miyanishi K, Takeda H, Islam A, Matsuoka N, Kubo M, et al. Phagocytic elimination of synapses by microglia during sleep. *Glia*. 2019

92. Hayashi Y, Koyanagi S, Kusunose N, Okada R, Wu Z, Tozaki-Saitoh H, et al. The intrinsic microglial molecular clock controls synaptic strength via the circadian expression of cathepsin s. *Sci Rep*. 2013;3:2744

93. Woolley CS, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J Neurosci*. 1990;10:4035-4039

94. Frankfurt M, Gould E, Woolley CS, McEwen BS. Gonadal steroids modify dendritic spine density in ventromedial hypothalamic neurons: A golgi study in the adult rat. *Neuroendocrinology*. 1990;51:530-535

95. Dantzer R. Cytokine-induced sickness behavior: Mechanisms and implications. *Ann.N.Y.Acad.Sci.* 2001;933::222-234

96. Tu TH, Nam-Goong IS, Lee J, Yang S, Kim JG. Visfatin triggers anorexia and body weight loss through regulating the inflammatory response in the hypothalamic microglia. *Mediators Inflamm*. 2017;2017:1958947

97. Jin S, Kim JG, Park JW, Koch M, Horvath TL, Lee BJ. Hypothalamic tlr2 triggers sickness behavior via a microglia-neuronal axis. *Sci Rep*. 2016;6:29424

98. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol*. 2011;29:415-445

99. Cai G, Dinan T, Barwood JM, De Luca SN, Soch A, Ziko I, et al. Neonatal overfeeding attenuates acute central pro-inflammatory effects of short-term high fat diet. *Frontiers in neuroscience*. 2015;8:446

100. Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, et al. Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest*. 2012;122:153-162

101. Douglass JD, Dorfman MD, Thaler JP. Glia: Silent partners in energy homeostasis and obesity pathogenesis. *Diabetologia*. 2017;60:226-236

102. Andre C, Guzman-Quevedo O, Rey C, Remus-Borel J, Clark S, Castellanos-Jankiewicz A, et al. Inhibiting microglia expansion prevents diet-induced hypothalamic and peripheral inflammation. *Diabetes*. 2017;66:908-919

103. Fonken LK, Lieberman RA, Weil ZM, Nelson RJ. Dim light at night exaggerates weight gain and inflammation associated with a high-fat diet in male mice. *Endocrinology*. 2013;154:3817-3825

104. Dorfman MD, Krull JE, Douglass JD, Fasnacht R, Lara-Lince F, Meek TH, et al. Sex differences in microglial cx3cr1 signalling determine obesity susceptibility in mice. *Nat Commun*. 2017;8:14556

105. Qu W, Johnson A, Kim JH, Lukowicz A, Svedberg D, Cvetanovic M. Inhibition of colony-stimulating factor 1 receptor early in disease ameliorates motor deficits in sca1 mice. *Journal of neuroinflammation*. 2017;14:107

106. Valdearcos M, Robblee MM, Benjamin DI, Nomura DK, Xu AW, Koliwad SK. Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function. *Cell Rep*. 2014;9:2124-2138

107. Gerber YN, Saint-Martin GP, Bringuier CM, Bartolami S, Goze-Bac C, Noristani HN, et al. Csf1r inhibition reduces microglia proliferation, promotes tissue preservation and improves motor recovery after spinal cord injury. *Frontiers in cellular neuroscience*. 2018;12:368

108. Reis WL, Yi C-X, Gao Y, Tschöp MH, Stern JE. Brain innate immunity regulates hypothalamic arcuate neuronal activity and feeding behavior. *Endocrinology*. 2015;156:1303-1315

109. Loncarevic-Vasiljkovic N, Pesic V, Todorovic S, Popic J, Smiljanic K, Milanovic D, et al. Caloric restriction suppresses microglial activation and prevents neuroapoptosis following cortical injury in rats. *PloS one*. 2012;7:e37215

110. Gao Y, Vidal-Itriago A, Milanova I, Korpel NL, Kalsbeek MJ, Tom RZ, et al. Deficiency of leptin receptor in myeloid cells disrupts hypothalamic metabolic circuits and causes body weight increase. *Molecular metabolism*. 2018;7:155-160

111. Rosin JM, Vora SR, Kurrasch DM. Depletion of embryonic microglia using the csf1r inhibitor plx5622 has adverse sex-specific effects on mice, including accelerated weight gain, hyperactivity and anxiolytic-like behaviour. *Brain Behav Immun*. 2018;73:682-697

112. Levkovitz Y, Mendlovich S, Riwkes S, Braw Y, Levkovitch-Verbin H, Gal G, et al. A double-blind, randomized study of minocycline for the treatment of negative and cognitive symptoms in early-phase schizophrenia. *J Clin Psychiatry*. 2010;71:138-149

113. Schmidtner AK, Slattery DA, Glasner J, Hiergeist A, Gryksa K, Malik VA, et al. Minocycline alters behavior, microglia and the gut microbiome in a trait-anxiety-dependent manner. *Transl Psychiatry*. 2019;9:223

114. Millan EZ, Ong Z, McNally GP. Paraventricular thalamus: Gateway to feeding, appetitive motivation, and drug addiction. *Prog Brain Res*. 2017;235:113-137

115. Ong ZY, Liu JJ, Pang ZP, Grill HJ. Paraventricular thalamic control of food intake and reward: Role of glucagon-like peptide-1 receptor signaling. *Neuropsychopharmacology*. 2017;42:2387-2397

116. Milanova IV, Kalsbeek MJT, Wang XL, Korpel NL, Stenvers DJ, Wolff SEC, et al. Diet-induced obesity disturbs microglial immunometabolism in a time-of-day manner. *Frontiers in endocrinology*. 2019;10:424

117. Tracy LM, Bergqvist F, Ivanova EV, Jacobsen KT, Iverfeldt K. Exposure to the saturated free fatty acid palmitate alters bv-2 microglia inflammatory response. *J Mol Neurosci*. 2013;51:805-812

118. Gosselin D, Skola D, Coufal NG, Holtman IR, Schlachetzki JCM, Sajti E, et al. An environment-dependent transcriptional network specifies human microglia identity. *Science*. 2017;356

119. Watkins LR, Hutchinson MR. A concern on comparing 'apples' and 'oranges' when differences between microglia used in human and rodent studies go far, far beyond simply species: Comment on smith and dragunow. *Trends in neurosciences*. 2014;37:189-190