REVIEW

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Microglia emerge as central players in brain disease

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There has been an explosion of new findings recently giving us insights into the involvement of microglia in central nervous system (CNS) disorders. A host of new molecular tools and mouse models of disease are increasingly implicating this enigmatic type of nervous system cell as a key player in conditions ranging from neurodevelopmental disorders such as autism to neurodegenerative disorders such as Alzheimer's disease and chronic pain. Contemporaneously, diverse roles are emerging for microglia in the healthy brain, from sculpting developing neuronal circuits to guiding learning-associated plasticity. Understanding the physiological functions of these cells is crucial to determining their roles in disease. Here we focus on recent developments in our rapidly expanding understanding of the function, as well as the dysfunction, of microglia in disorders of the CNS.

Once virtually ignored, microglia, the resident immune cells of the CNS, have recently taken center stage in research for their roles in CNS health and disease. In the healthy brain and spinal cord, microglia, which represent approximately 10% of CNS cells, form a near-regular three-dimensional lattice in which each microglial cell occupies unique territory. Microglia are highly ramified cells that have a multitude of fine, exceptionally motile processes that continuously survey the parenchymal environment. Through this surveillance, microglia detect diverse extracellular signals, and consequently, transduce, integrate, and respond to them to maintain brain homeostasis. Researchers are increasingly coming to appreciate the variety of microglial responses, recognizing that many of the response states are distinctly different from CNS inflammation. Thus, in contrast to the roles they have had attributed to them in the past, microglia are now known to be active participants in brain function and dysfunction.

The majority of recent information gained about the roles for microglia come from applying powerful new technologies in the mouse and in mouse models of disease. Investigations of microglia in humans are becoming more frequent, but more selective and specific biomarkers of microglia states are required to determine whether biological findings in the mouse are applicable to humans, and whether therapeutic approaches targeted to microglia in mouse models of disease predict treatment response in human diseases.

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Scientific interest in the role of microglia in disease is growing at an ever-accelerating rate. In this article, we first describe the current understanding of the ontogeny of microglia and the cells' physiological roles in the CNS, which is based largely on research using animal models. We then discuss the emerging roles for microglia in CNS diseases (**Table 1**). Finally, we speculate on the potential therapeutic opportunities that may come from targeting the aberrant functions of microglia in disease, and describe some of the hurdles that must be overcome.

Ontogeny, development, and maintenance of microglia

Elegant lineage-tracing studies in mice have established that microglia are derived from yolk-sac progenitors that express the transcription factor RUNX1 and the receptor tyrosine kinase c-Kit, also known as CD117, but that do not express CD45, a pan-leukocyte marker protein[1]. These progenitors migrate through the bloodstream to the developing CNS, starting at embryonic day 8.5 and continuing until the blood–brain barrier is formed[2,3]. Microglia may be considered to be similar to tissue-resident macrophages in non-CNS tissues, but it has recently been determined that microglia are the only myeloid cells that are derived solely from yolk-sac precursors under normal conditions[4,5]. Lineage-specific genes, such as *Pu.1* and *Irf8*, define the microglial transcriptional network and distinguish it from that of tissue-resident macrophages[1,6–8]. Microglia, which are parenchymal cells in the CNS, are also distinct from perivascular, meningeal, and choroid plexus macrophages, although transcriptome analysis has revealed a striking overlap in the transcriptional profiles between microglia and perivascular macrophages, much more so than between microglia and circulating monocytes[9].

As the CNS develops and matures, microglia must progressively adapt to take on distinct sets of physiological functions. Examination using RNA sequencing and epigenomic analyses of the dynamics of the transcriptional repertoire from the yolk sac to the adult in mice has revealed three major developmental states: early (less than E14), pre-microglia (E14 to a few weeks postnatal), and adult microglia (more than a few weeks)[10]. Single-cell analysis identified a stepwise developmental

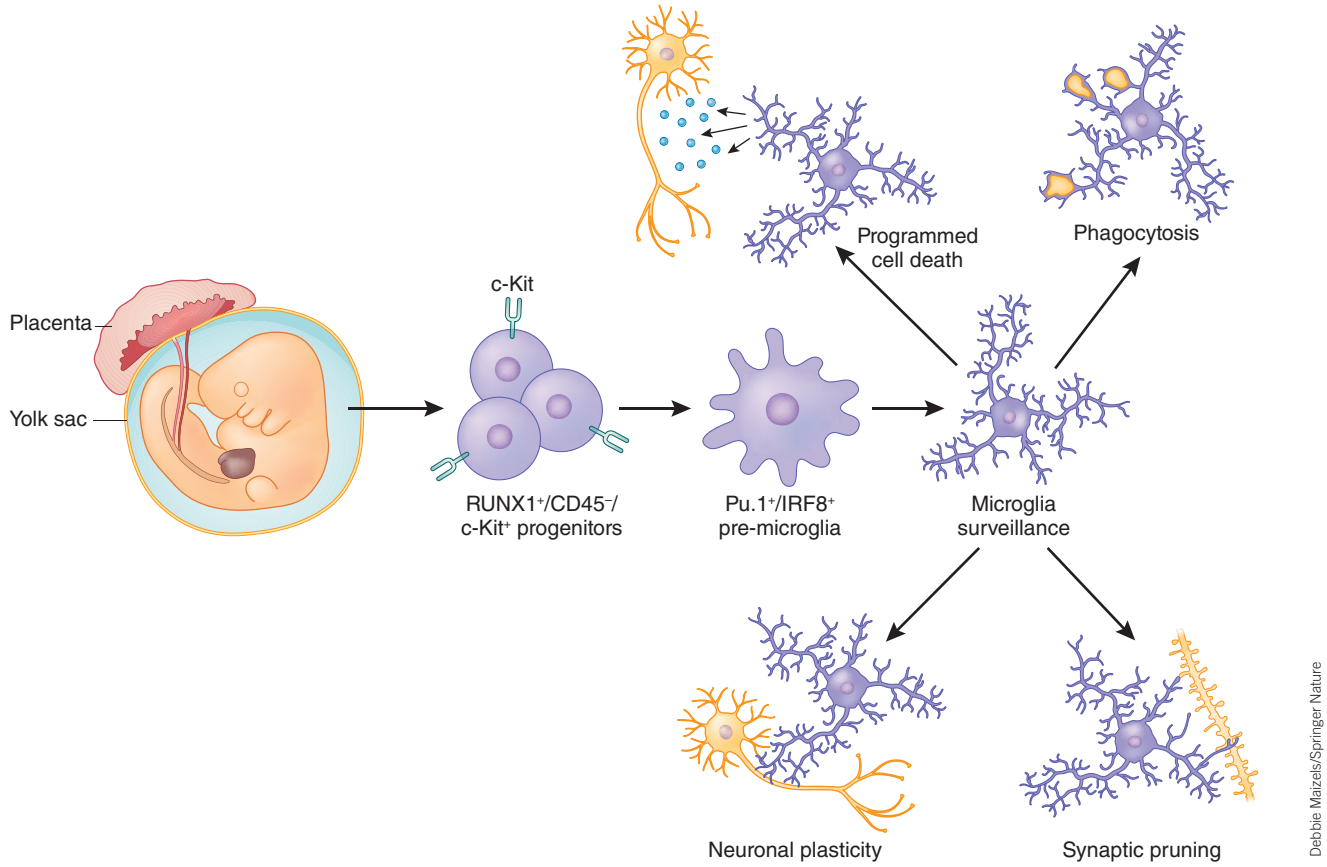

Figure 1 Ontogeny of microglia and physiological roles in CNS development, homeostasis, and plasticity. Microglia develop from myeloid progenitors in the yolk sac that express the transcription factor RUNX1 and the receptor tyrosine kinase c-Kit, also known as CD117, but not CD45, and enter the CNS during early embryonic development (embryonic day 8.5 in mice). Microglia have normal roles in brain development and CNS homeostasis, including programmed cell death and clearance of apoptotic newborn neurons, as well as pruning developing axons and synapses. Later in development and into adulthood, microglia processes are highly motile and continually survey their local environment, contacting neurons, axons, and dendritic spines. Microglia have diverse physiological roles, including regulating neuronal and synaptic plasticity.

program in which individual microglial cells shift their regulatory networks in a coordinated manner, such that, as a group, microglia are synchronized with neuronal development. It is hypothesized that the different stages of microglia development utilize distinct pathways for processing homeostatic neurodevelopmental signals from the environment, balanced by the need to maintain the capacity for appropriate immune responses. Although there is some commonality among microglia in ontogeny and developmental programs, hierarchical clustering analysis[11] indicates that there may be microglial subpopulations with distinct transcriptomes. This concept builds on findings of functional, morphological subpopulations of microglia in different CNS regions[12,13].

The original pool of yolk-sac-derived cells is the source of myeloid cells in the healthy CNS, and the maintenance of microglia does not dependent on circulating monocytes[4,14,15]. Thus, when new microglia are required, these must be generated by self-renewal from local pools that persist over long periods. In the healthy adult, individual microglia are long-lived, turning over at a rate of 0.05% of cells per hour[16]. Maintenance of the microglia population depends on the continuing activation of the receptor for colony-stimulating factor 1 (CSF-1R), which is also essential for microglia and macrophage development[2,17]. The endogenous ligands produced in the CNS for CSF-1R–CSF-1 and interleukin (IL)-34 (ref. 18)—must be released continuously, because pharmacological blockade of CSF-1R leads to rapid depletion of microglia[19].

The physiological roles of microglia in development and synaptic plasticity

Microglia not only respond when things go wrong, but they also instruct the form and function of the healthy CNS (**Fig. 1**). Numerous studies have demonstrated that microglia have a number of physiological, noninflammatory functions that are crucial for CNS development and for regulating neuroplasticity in the adult[20–22]. Given that microglia are present in the CNS from the mid-embryonic stage onward, they are positioned to carry out roles in many aspects of the subsequent development of the CNS.

Microglia surveillance and monitoring

Two-photon imaging studies *in vivo* in mice expressing green fluorescent protein specifically in microglia have revealed that these cells have highly dynamic processes and continually survey their local environment[23,24]. It is estimated that resident microglia scan the entire volume of the brain over the course of a few hours[24], suggesting that they have homeostatic functions in the healthy brain. The fine processes of microglia continuously contact neurons, axons, and dendritic spines. Moreover, process motility can change dramatically in response to extracellular stimuli, including neuronal activity[25,26] and neurotransmitters[26]. ATP is a major stimulus of microglia, and microglial processes are rapidly attracted to the source of ATP release[23], which is a feature that can be manipulated by glutamate-receptor agonists in rodent models *in vitro* and *in situ*[6–8].

Table 1 Microglial genes and pathways implicated in health and disease

Health and disease	Genes and/or pathways of interest	References
Synaptic pruning	C3R, C1A, CR3, and CX3CR1	30,33
Neuronal programmed cell death	CR3, TAM-receptor kinases (AXL, Mer), DAP12, NGF, and TNF	38,40–42
Synaptic plasticity	Tlr4, TGF-β, CX3CR1, BDNF, and TNF	44,48,50,57,58,162
Nasu–Hakola disease	TREM2	84
Rett syndrome	MECP2	71–73
Alzheimer's disease	TREM2, DAP12, APOE, APOJ, CD33, CR1, C1q, C3, and classical-complement cascade	97–114, 117,118,163–165,
Frontal temporal dementia	Progranulin and C1qA	33
Neuropathic pain	$P2RX_4$, P2RX7, P2RY12, BDNF, DAP12, IRF5, and IRF8	129–140

Glutamate-receptor agonists can stimulate ATP-mediated microglial-process outgrowth in rodent models *in vitro* and *in situ*[27–29]. Although the functional significance of microglial process motility and local surveillance remains elusive, ATP is likely one of many signals that mediates microglia–neuron communication and so could influence neuronal and synaptic function in health and disease.

Developmental synaptic pruning

During development, microglia sculpt immature neuronal circuits by engulfing and eliminating synaptic structures (axons and dendritic spines) in a process known as synaptic pruning[30–32]. Electron microscopy and high-resolution *in vivo* engulfment assays have revealed pre- and postsynaptic structures inside microglial lysosomes in the mouse visual system, hippocampus, and other brain regions during critical periods of synaptic refinement. The disruption of microglia pruning leads to sustained defects in synaptic development and wiring abnormalities[31]. Intriguingly, in the mouse visual system, this pruning is dependent on neuronal activity and sensory experience[30,31], wherein microglia preferentially engulf less active presynaptic inputs. This raises questions about how specific synapses are recognized and molecularly targeted by microglia for pruning.

One group of molecules involved in pruning is the classical-complement cascade[22,31,33,34]. In the innate immune system, complement proteins are 'eat me' signals that mark apoptotic cells and pathogens for removal by circulating macrophages that express C3 receptors (CR3/CD11b)[35]. In the healthy developing murine brain, C3 and C1q are widely expressed and localize to subsets of immature synapses[33]. Microglia, the only CNS cell type that expresses a C3 receptor (CR3), phagocytose these synaptic inputs through the same C3–CR3 signaling pathway as is used by the innate immune system[31]. Mice deficient in C1q, C4 (ref. 36), C3, or CR3 have sustained defects in synaptic connectivity, suggesting that the healthy brain requires the activation of the classical-complement cascade. Fractalkine, the neuronal CX3CR1 ligand CX3CL1, has also been implicated in microglial synaptic pruning; mice that lack this receptor have markedly fewer microglia during early postnatal development than those that express the receptor, and also show defects in synaptic maturation and hippocampus refinement[32].

There are likely other molecules that work in concert with complement and fractalkine to prune specific synapses and to ensure that pruning occurs at the right time and place. It is possible that different mechanisms regulate pruning in different contexts, including across brain regions and stages of development. Aberrant pruning during critical developmental periods could contribute to neurodevelopmental disorders, such as autism and schizophrenia, as discussed later.

Driving neuronal programmed cell death

Given that microglia are phagocytic cells, one role in CNS development is to engulf and clear neurons that die as a result of programmed cell death, a process that eliminates excess neurons generated as part of normal development and ongoing neurogenesis in the adult[37,38]. Crucial for the removal of the cellular corpses in adult neurogenesis are the TAM-receptor kinases AXL and Mer[39]. But, microglia are not simply waste scavengers; they are also active neuronal exterminators, driving programmed cell death by inducing apoptosis of neurons without provoking inflammation. Microglia may drive neuronal apoptosis through the release of superoxide ions[40], nerve growth factor[41], or tumor necrosis factor (TNF)[42]. Microglia-induced neuronal apoptosis is dependent on the cell-surface receptor CR3, the activation of which initiates intracellular downstream signaling through DNAX activation protein of 12 kDa (DAP12) (also known as TYROBP), ultimately triggering the release of the neurotoxic factors[43]. In addition to microglia having a role in the initiation of programmed neuronal death, they may also be involved in expediting the processes leading to death in neurons that are induced by other mechanisms to be vulnerable[44]. In physiological conditions, microglial phagocytosis is coupled to apoptosis through 'find-me' signals, such as adenosine triphosphate (ATP), which are released by apoptotic cells, and so appropriate coupling of phagocytosis to apoptosis is physiologically important[25]. Moreover, it has been found that in the healthy CNS, only a small proportion of microglia are in the process of phagocytosing at a given time, which suggests that there may be large potential for increasing the number of microglia performing phagocytosis, a mechanism that could be targeted in the impaired CNS[25].

Microglia in synaptic plasticity in the adult

Unstimulated microglia in the healthy CNS have no demonstrable role in ongoing, basal synaptic transmission or in short-term plasticity, although the stimulation of microglia, for example, by activating TLR4, may affect synaptic function[45]. Rather, microglia come into play during the processes of activity-dependent, long-term synaptic plasticity. Such persistent synaptic plasticity is commonly understood to be a fundamental cellular underpinning of learning and memory[46], and one that may manifest as either an increase or a decrease in synaptic efficacy, commonly referred to, respectively, as long-term potentiation and long-term depression. Learning and memory are also highly dependent on the proliferation and development of new neurons—a process called neurogenesis[47]—which occurs in the healthy brain as well as during development[48]. The cumulative body of evidence from numerous laboratories using a range of approaches suggests that microglia are crucial regulators of activity-triggered synaptic plasticity[49–53], adult neurogenesis[54–56], and learning and memory[49,57,58]. Changes in microglial number or function during development, for example, through the deletion of transforming growth factor (TGF)-β in the CNS[51,59] or by knocking out CX3CR1 (ref. 49), results in aberrations in neuroplasticity in adulthood. But more importantly, eliminating microglia in the adult or blocking the cells'

ability to make brain-derived neurotrophic factor (BDNF) leads to impaired synaptic plasticity and learning and memory[58].

Roles in CNS pathology and disorders

Our growing understanding of the pleiotropic physiological roles of microglia in the developing and mature CNS suggests that aberrations in these normal functions of microglia may contribute to disease processes in the CNS (**Fig. 2**).

Microglia 'activation' and plasticity

Microglia are dynamic cells that respond subtly, and sometimes grossly, to a near endless variety of environmental cues. A hallmark of microglial responsivity is the cells' ability to alter their own morphology, with or without proliferation. Indeed, differences in microglial morphology were described by Pio del Rio Hortego, who discovered microglia more than 100 years ago and correlated their morphology with pathological transformation[60]. But, in fact, the morphological changes indicate only that the microglia have detected a change in homeostasis; they do not specify a particular response state or type of activity in any given CNS disease.

Previously, the responsivity of microglia was conceptually shoehorned into a monolithic state—activation—that consisted of linear progression through a small number of states defined morphologically from ramified to ameboid. Attempts have even been made to fit microglia responsivity into a bimodal scheme: the M1 and M2 states, borrowed from the now-defunct classification of macrophages[61]. Similarly to macrophages, modern transcriptome profiling of microglia in mice has shown that the response phenotypes fail to conform to M1/M2 patterns[62–64]. New approaches that examine the transcriptomic, proteomic, and epigenomic characteristics of microglia in specific contexts are beginning to reveal consistent, discrete responsivity patterns in health and disease.

Microglia in autism and developmental disorders

A role for microglia in autism and developmental disorders has been speculated about for some time[65], but direct evidence has been lacking. New insights into the functions of microglia in the healthy developing brain in the mouse are providing a new perspective on this potential link. As microglia colonize the brain during early embryonic development, environmental and/or genetic perturbations could alter microglial development, synaptic pruning and surveillance, or other functions that could directly or indirectly contribute to the pathobiology of neurodevelopmental and neuropsychiatric disorders.

Several lines of evidence from human studies suggest that microglia are dysfunctional in autism-spectrum disorders (ASD), a complex group of neurodevelopmental disorders characterized by impairments in social and verbal communication and other characteristic behaviors. Postmortem studies have revealed altered microglial counts, morphology, and neuronal interaction in brains from individuals with autism, notably, in regions such as the dorsolateral prefrontal cortex that control executive functions[66–68]. Moreover, genome-wide transcriptional analyses of postmortem brain tissue have found that brains from some individuals with autism have altered expression of microglia-specific genes, including markers related to the inflammatory state[69–71]. Intriguingly, a human brain-imaging study using positron-emission tomography (PET) with the radiotracer [^{11}C](*R*)-PK11195, a ligand for the translocator protein (TSPO), which is expressed in microglia and astrocytes, has revealed increased [^{11}C](*R*)-PK11195 binding in the brains of young adults with autism[70], a finding consistent with postmortem studies. However, [^{11}C](*R*)-PK11195, although informative, is not specific for microglia and may also be a more general indicator of gliosis and neuroinflammation. Although these studies indicate that there are changes in microglial numbers or state in some individuals with autism, it will be important to determine whether the microglia are simply responding to aberrant changes in brain environment, or whether they have a contributing role.

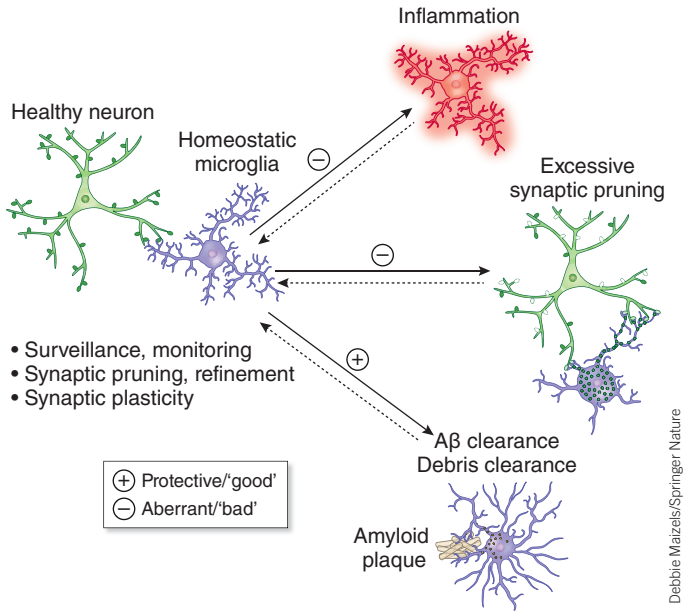

Figure 2 Microglia states in health and disease. Microglia have complex roles that are both beneficial and detrimental to disease pathogenesis, including engulfing or degrading toxic proteins (i.e., amyloid plaques) and promoting neurotoxicity through excessive inflammatory cytokine release. Aberrations in microglia's normal homeostatic functions such as surveillance, synaptic pruning, and plasticity may also contribute to excessive synapse loss and cognitive dysfunction in AD and other diseases.

Although human genetic studies do not implicate genes specifically expressed by microglia as having a causative role for ASD, microglia could aberrantly respond to insults that originate in neurons or other cells (i.e., astrocytes), thereby contributing to neuronal and circuit dysfunction in autism. This was recently demonstrated in a mouse model of Rett syndrome[72], a neurodevelopmental disorder usually caused by mutations in methyl-CpG binding protein 2 (*MECP2*)[73,74]. When microglia–synapse interactions were examined before, during, and after the onset of phenotypic and synaptic regression in the visual system of mice lacking *Mecp2,* microglia were shown to excessively engulf synapses at end stages of disease. However, this effect was independent of microglia-specific loss of MECP2 expression, suggesting that microglia aberrantly respond to the loss of MECP2 in neurons or other cell types. These results also suggest that synaptic pruning could become dysregulated or overactive and so could contribute to aberrant synapse loss and dysfunction in ASD and neurodevelopmental disorders, including schizophrenia[36].

Environmental factors could also alter microglia function, thus influencing brain development and synaptic connectivity. Because microglia respond to inflammatory signals, environmental conditions that cause systemic or local inflammation during the developmental critical period may have consequences throughout life. This could explain how prenatal infection and maternal immune challenge seem to increase the risk of autism and schizophrenia in offspring. Indeed, maternal immune activation in pregnant rodents induces robust behavioral deficits in offspring. A single maternal challenge with viral or bacterial components (i.e., PolyIC) during embryonic development (E9–12) results in behavioral changes in the offspring, including decreased social behavior, increased repetitive behaviors, increased anxiety, and altered ultrasonic vocalization[75].

A second challenge (i.e., stress) later in development was shown to increase the severity in mice of some maternal-immune-activation-related behavioral defects that are related to anxiety, memory, and cognition function[76].

Furthermore, a recent study using genome-wide chromatin and expression profiling throughout development identified several temporal stages of microglial development that are sensitive to both genetic and environmental perturbations, including alterations in the microbiome or prenatal immune activation[10]. The developmental and behavioral deficits observed in maternal-immune-challenged offspring are mediated by interleukin (IL)-6, a proinflammatory cytokine released from macrophages and T cells to elicit an immune response, and are accompanied by long-term cytokine dysregulation[77,78]. Microglia could modulate the maturation or function of neurons and synaptic function through the release of inflammatory cytokines and molecules, such as interleukins or TNF, which also regulates synaptic maturation and plasticity.

Thus, genetic or environmental disruption of microglial development or function could have functional and behavioral consequences and may be relevant to ASD and other disorders. In rodents, perturbations in microglia[79], including genetic deletion of microglia-specific receptors (*Cx3cr1* or *Dap12*), result in defects in the outgrowth of dopaminergic axons in the forebrain and the laminar positioning of subsets of neocortical interneurons. Moreover, defects in synaptic function and connectivity, including altered pruning of cortical synapses, are thought to underlie autism and schizophrenia[80,81]. In support of this idea, *Cx3cr1*-knockout mice, which have impaired synaptic pruning, also have sustained defects in social behavior and functional long-range connectivity[82]. In addition, genetic disruptions in myeloid cells contribute to behavioral abnormalities in mice. Compulsive overgrooming behavior in mice with mutations in *Hoxb8*, a homeobox transcription factor, is mediated by bone-marrow-derived microglia, the only cell in the brain that expresses HOXB8 (ref. 83). The HOXB8 cell lineage specifically gives rise to brain microglia. Transplantation of bone marrow from wild-type mice into irradiated HOXB8 mice rescued the excessive-grooming and hair-loss phenotypes, which suggests that microglia contribute to core symptoms of the disorder[83]. Finally, in humans, homozygous mutations in triggering receptor expressed on myeloid cells 2 (*TREM2*)—which encodes an innate immune receptor expressed on microglia and myeloid cells—is associated with Nasu–Hakola disease, a rare disease characterized by multiple bone cysts, leukoencephalopathy, and early mid-life dementia and neurodegeneration[84], findings that provide evidence that microglia are important for cognitive function[85].

Understanding when and where microglia become dysfunctional in autism and other neurodevelopmental and neuropsychiatric disorders will be crucial for understanding how microglia influence circuits and brain regions relevant to these disorders. Brain development shows distinct sexual dimorphism[86], in which sex differences in microglia function may have a key role[87,88] (see **Box 1**). Such sex differences in microglial function could underlie, at least in part, differences observed in susceptibilities to and outcomes of neurodevelopmental and neuropsychiatric disorders in males and females[89].

Microglia in neurodegenerative disorders

Reactive gliosis and neuroinflammation are hallmarks of Alzheimer's disease (AD), a progressive neurodegenerative disorder that is the most common cause of dementia, and other neurodegenerative diseases, including Parkinson's disease, amyotrophic lateral sclerosis (ALS), and frontal temporal dementia[90]. Long considered to be secondary events to neurodegeneration, microglia-related pathways have been identified by emerging genetic and transcriptomic studies as central to AD risk and pathogenesis[91–94]. Large-scale genome-wide association studies (GWAS) have identified more than 20 loci that are linked to AD, many of which are expressed or exclusively expressed in microglia or myeloid cells[95,96].

Among these risk genes for AD is *TREM2*. Individuals heterozygous for the rare *TREM2* variant R47H are at a significantly increased risk for AD, and several other *TREM2* variants have been associated with AD, frontal temporal dementia, and possibly, Parkinson's disease, indicating that the dysfunction of microglia (or infiltrating myeloid cells) is important in neurodegenerative disorders[97]. Additionally, the level of soluble TREM2 in cerebrospinal fluid is increased in very early stages of AD, i.e., in those diagnosed with mild cognitive impairment[98]. However, the underlying biology and relevant ligands remain poorly understood. In the immune system, TREM2 mediates inflammatory and phagocytic signaling in immune cells through engagement of its co-receptor DAP12 (ref. 99). Mutations that impair TREM2 signaling could thus contribute to AD pathogenesis by affecting several crucial functions of microglial phagocytosis, microglial survival, neuroinflammation, and other processes[97,100].

Microglia surround amyloid plaques in human AD brains and in animal models of AD. They have complex roles that are both beneficial and detrimental to AD pathogenesis, including engulfing or degrading amyloid plaques and promoting neurotoxicity through excessive inflammatory cytokine release[90] (**Fig. 2**). Failure to clear apoptotic cells, cellular debris, and toxic proteins, such as beta-amyloid (Aβ) can contribute to inflammation and neurodegeneration. Recent studies have reported fewer microglia or monocytes surrounding Aβ plaques in the absence of TREM2, with varying—and sometimes contradictory—effects on plaque burden in AD models[101–103]. Some studies report the amelioration of plaques, inflammation, and AD pathology in the absence of TREM2, whereas others suggest that TREM2 has a beneficial role in AD. These differences are likely due to the specific animal model and also the time points studied, because TREM2 deficiency in the same model has been shown to have distinct roles in early as opposed to late stages of amyloid pathology[104]. More research is needed to elucidate the relevant mechanisms. New data suggest that apolipoprotein E (APOE) binds TREM2 to regulate efficient clearance of apoptotic cells and debris[105]. Interestingly, a recent unbiased screen identified apolipoproteins, including APOE and APOJ, as putative ligands of TREM2, and suggests that they can facilitate the uptake of Aβ and Aβ–APOE complexes by microglia *in vitro*[106]. Because the *APOE* genotype has the strongest association of the apolipoproteins with AD risk, it will be crucial to understand the underlying mechanisms that govern interactions between TREM2, APOE, and other microglia-specific pathways to increase risk and AD pathogenesis. Interestingly, cell-surface TREM2 can be cleaved by a protease, and soluble TREM2 levels are decreased in the cerebrospinal fluid of individuals with early AD or frontal temporal dementia[98,107,108], identifying it as a potential biomarker. Although much of the field has focused on plaque clearance and inflammation during the later stages of AD, TREM2 could also have earlier, homeostatic functions related to cognition[97], because mutations in *TREM2* or *DAP12* cause progressive cognitive impairment[109,110]. Whether TREM2 deficiency impairs synaptic or cognitive functions in AD and other neurodegenerative diseases is an important question for investigation.

Several other genes specifically expressed or enriched in microglia have emerged as genes conferring susceptibility to AD, including *CD33*, a transmembrane receptor on myeloid cells, which has been shown to have a role in Aβ clearance and neuroinflammation[111,112]. Members of the classical-complement cascade, APOJ (clusterin), and complement receptor 1 (CR1)[113,114] have also been linked to late-onset AD. The complement cascade is substantially upregulated and activated in the AD-affected brain; however, the link between AD and complement

Box 1 Sex differences in microglia

Heterogeneity in human brain structure and function has been widely acknowledged for some time. However, the question of whether such differences can be directly linked to sex chromosomes and/or influenced by sex hormone is still being debated, despite growing evidence showing that sex can indeed be a determinant of structural and functional differences that exist in the brain (see review in ref. 86). Observations from the late 1980s had implicated sex hormones as modulators for neuronal and astrocytic developmental programming[153,154]. It was not until later that sex differences were investigated in microglia and for their possible involvement in disease[155]. So far, the influence of sex differences on microglia function has been linked not only to development[156], but also to many CNS perturbations, including traumatic injury, ischemia, and stress[157–160], although exactly what mechanisms are involved is still unclear. Some insight into the impact that sex has on microglial functional divergence has recently been revealed in pain signaling and in the development of male copulatory behavior. Previously, it was shown that pain hypersensitivity after a peripheral-nerve injury in male mice involves signaling from spinal-dorsal-horn microglia[151]. Strikingly, recent findings have revealed that female mice with the same injury do not require microglia; rather, in females, the pain signal is maintained by adaptive immunity, and this profound cell-type difference in how males and females maintain pain hypersensitivity is largely hormonal[139]. In embryonic development, androgen levels are crucial for the masculinization of the preoptic area (POA) and, ultimately, adult male copulatory behavior. Lenz *et al.*[161] have shown that microglia inhibition in males prevented POA masculinization and affected adult behavior, whereas activating microglia with estradiol in females induced the masculinization of the POA. These recent studies demonstrate that sex can be a strong contributor to both normal and pathological functions of microglia in mammals, a finding that has far-reaching implications on how we interpret physiological responses, and ultimately, develop therapeutic strategies.

was largely thought to be related to plaque clearance and neuroinflammation associated with neurodegeneration[90]. However, recent studies implicate microglia, complement, and other immune-related pathways as early mediators of synaptic dysfunction in the absence of plaques and overt inflammation.

Synapse loss is an early hallmark of AD and other neurodegenerative disorders and is considered a strong correlate of cognitive decline[115,116]. Complement proteins (C1q, C3) are upregulated in several AD mouse models in the hippocampus and vulnerable brain regions, and they associate with synapses before overt plaque deposition, causing enhanced engulfment of synaptic elements[117]. Moreover, genetic and antibody-mediated inhibition of C1q, C3, and/or CR3 rescues synapse loss and Aβ-mediated synaptic dysfunction, suggesting that microglia contribute to early synapse loss and dysfunction in a pre-plaque model of AD[117]. Taken together, these findings suggest that the same pathway that prunes excess synapses in development are inappropriately activated and mediate synapse loss in AD[117,118].

Could this microglial pruning pathway be a common mechanism underlying synapse loss and dysfunction in CNS neurodegenerative disease? In support of this idea, deletion of the frontal temporal dementia gene, progranulin, in mice results in neurodegeneration and behavioral phenotypes, as well as complement activation and enhanced synaptic pruning, preferentially for inhibitory synapses in a mouse model of FTD. The phenotypes can be rescued by deleting C1q A chain (C1qA)[34]. Moreover, in glaucoma, a neurodegenerative disease that involves the selective loss of retinal ganglion neurons[33], the complement cascade is upregulated and targets vulnerable synapses very early in disease. The genetic deletion of *C1qa* in the DBA2J glaucoma mouse model protects retinal ganglion cell (RGC) neurons and prevents disease progression[119] and synapse loss[120]. Complement and microglia were also recently shown to mediate synapse loss in a model of virus (West Nile virus)-induced memory impairment[121]. Thus, understanding the signals that trigger microglia and complement to prune vulnerable circuits could provide important insights into potential new therapeutic targets.

Another subset of glia, astrocytes, has been shown in recent years to activate into at least two different states that depend on the mode of the initiating injury[122]. Inflammatory insult induces a so-called A1 astrocyte, whereas ischemia induces a different phenotype, called A2. New research has also revealed that aberrant microglia signaling can induce astrocytes, CNS glia, and key regulators of synapse function and plasticity and brain homeostasis[123–125] to transform into A1 astrocytes that were shown to be powerfully neurotoxic[126]. C1q was one of several factors (in addition to TNF and IL-1α) released by activated microglia that trigger the conversion of homeostatic astrocytes into neurotoxic reactive astrocytes that could contribute to neurodegeneration as well as to the impairment of several synaptic and neuronal functions, including synaptic pruning. Indeed, A1 astrocytes are abundant in various human neurodegenerative diseases, including Alzheimer's, Huntington's, and Parkinson's diseases, ALS, and multiple sclerosis[126,127]. What remains to be seen and will be interesting going forward is to investigate to what degree neurodegeneration can be augmented by targeting both astrocyte and microglial activation states.

Recently, it has been found in a mouse model of AD (the 5XFAD model) that driving γ-frequency oscillations reduces amyloid-plaque load[128]. Transcriptome changes in microglia suggested that microglial phagocytosis of Aβ is enhanced by γ-entrainment. Whether synaptic loss and cognition are improved by this potentially exciting approach to indirectly affect microglia function remains to be determined. Given that the A2 state of reactive astrocytes may promote survival and repair[126], it may be that synaptic and cognitive function are improved through the coordinated actions of entrained microglia together with astrocytes in the A2 state.

Microglia in neuropathic pain

Increasingly, studies in rodents[129] and humans[130] are implicating microglia in peripheral neuropathic pain, a debilitating and prevalent condition arising from damage to peripheral sensory nerves. Peripheral-nerve injury has been found to elicit a series of changes that drives a stereotypic microglial response in the sensory-processing region of the spinal cord[129]. Central to the pain hypersensitivity is transcriptional upregulation of the purinergic receptor, P2X4, in the microglia (**Fig. 3**). The activation of P2X4Rs by ATP released from spinal interneurons[131] initiates signaling within the microglia, leading to a release of BDNF that subsequently acts on pain-transmitting neurons to suppress inhibition. This lack of inhibition—disinhibition—ultimately results in pain hypersensitivity. The pain hypersensitivity process can be conceptualized as an exaggeration of the physiological action of microglia-derived BDNF in learning and plasticity[58], but in the context of a different neuronal network: one in which potentiating the output leads not to learning, but to enhanced pain.

Additional purinergic receptors, expressed by spinal microglia and P2X7 and P2Y12 receptors, have been implicated in pain hypersensitivity caused by peripheral-nerve injury[130,132–134]. Whether these receptors also act through BDNF signaling and disinhibition is currently an open question.

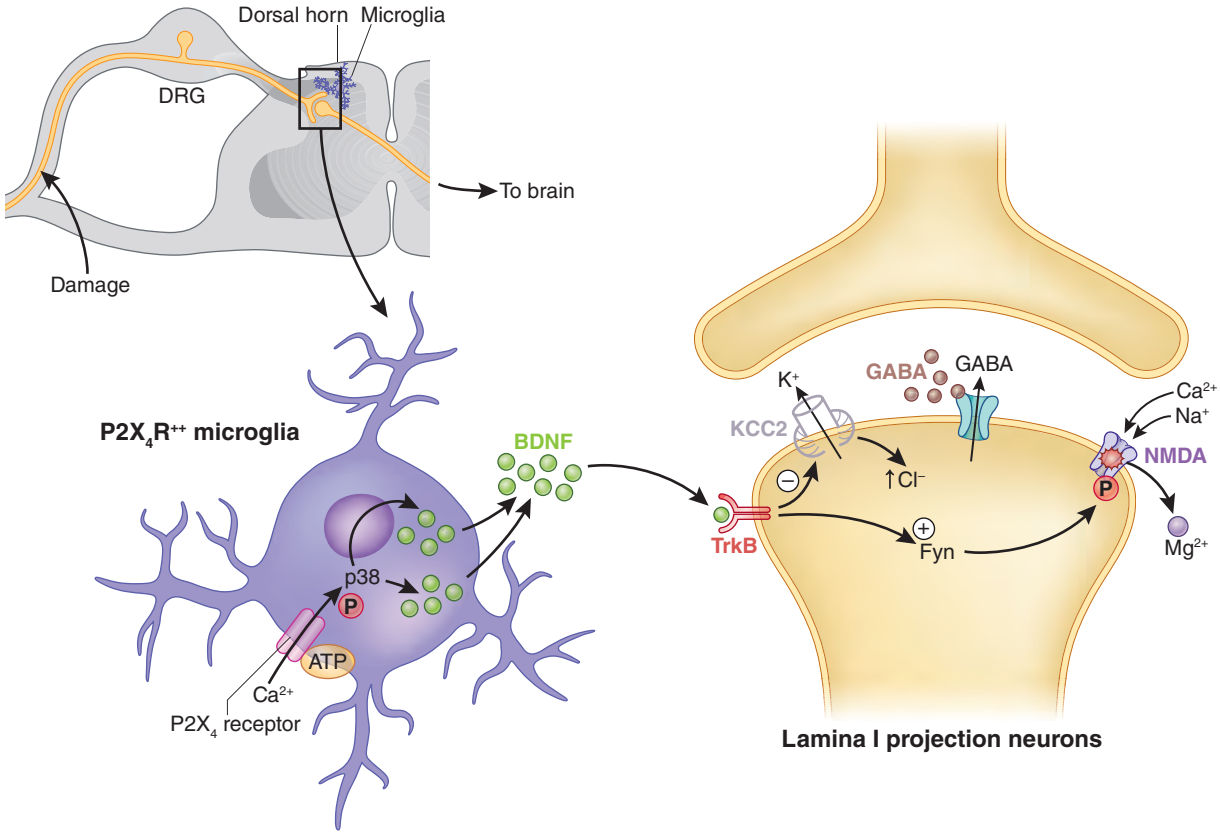

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Figure 3 Microglia–neuron interactions in the spinal cord are crucial to pain hypersensitivity induced by peripheral-nerve injury in males. Damage to peripheral sensory nerves, which in humans may lead to peripheral neuropathic pain, causes microglia in the sensory part of the spinal cord to respond by upregulating expression of the purinergic $P2X_4$ receptor[129]. Activating microglial $P2X_4$Rs initiates a signaling cascade, including calcium influx, that ultimately leads to hyperexcitability of neurons in spinal lamina I through the downregulation of KCC2 (disinhibition[151]) and the upregulation of *N*-methyl D-aspartate receptors (NMDARs) (enhanced excitation[152]). The involvement of microglia in nerve-injury-induced pain hypersensitivity is sexually dimorphic, occurring only in males. In females, the upregulation of $P2X_4$-receptor expression is blocked, and adaptive immune cells drive hyperexcitability of neurons in ascending pain pathways. DRG, dorsal root ganglion.

Peripheral-nerve injury elicits a proliferative response with profound morphological changes of spinal microglia, as well as pain hypersensitivity. If pain hypersensitivity is dependent upon morphological changes and proliferation, these should follow mechanistically step by step. The pain hypersensitivity is dependent upon DAP12 (ref. 135), the transcription factors IRF8 and IRF5 (refs. 136,137), P2X4Rs[138], and microglial-derived BDNF[139,140]. But the proliferative response and morphological changes are independent of all of these, which demonstrates that morphological changes can be separate from, and not causal for, changes in microglia function.

Surprisingly, the involvement of microglia in neuropathic pain hypersensitivity is sexually dimorphic: pain depends on microglial signaling only in males[139]. Despite this profound difference in cellular dependency, the degree of pain hypersensitivity in female mice is as great as that in male mice. Even more strikingly, the microglial proliferation and morphological changes induced by nerve injury are indistinguishable in females and males. But in females, the transcriptional upregulation of P2X4Rs is blocked by a mechanism dependent on adaptive immune signaling to microglia[139]. Although the details of this signaling remain to be worked out, this is a clear demonstration of sex differences in a disease process for which microglia are crucial (see also **Box 1**).

Therapeutic opportunities by targeting microglia

The recent advances in our understanding of where, when, and how the physiological functions of microglia—such as pruning, regulating plasticity, and neurogenesis—are dysregulated in CNS disorders are opening up possibilities for new opportunities for therapeutic interventions and disease diagnosis. Targeting the mechanisms that are dysregulated may arrest or reverse neurodevelopmental and neurodegenerative disorders in which microglia have a role. Moreover, microglia-based diagnostic approaches, such as biomarkers—for example, soluble TREM2 or complement components in cerebrospinal fluid—or neuroimaging approaches with probes toward microglia signaling pathways, may allow for the detection of at-risk individuals much earlier than is possible with biomarkers directed at neuronal dysfunction. Such microglia-based biomarkers may also permit the monitoring of disease progression and recovery. Given the sexual dimorphism in microglial function and dysfunction and the sex bias in CNS disorders with microglial pathology (including ASD and AD), the development of microglia-directed biomarkers and therapeutics will need to address pathways and targets that may differ in men and women.

To develop new therapeutics based on targeting microglia, a deeper dive into the mechanisms underlying microglia function and dysfunction is needed. Given the complexity and diverse functions of microglia in health and disease, there is a crucial need for new biomarkers that relate to specific microglial functional states (for example, abnormal phagocytosis of synapses as opposed to plaques; the inflammatory compared to the homeostatic states). Newly developed approaches for single-cell

RNA sequencing and the profiling of rodent and human microglia, such as DropSeq[141], are likely to provide important new insights and help to identify region- and species-specific alterations in microglia both in animal models and human disease[142]. New approaches to modeling disease with human induced pluripotent stem cell (iPSC) microglia also present an exciting and promising area for future development[143–145], with the potential to model microglia disease and develop the biomarkers and therapeutics. Studying microglia *in vitro* has been challenging, because removing microglia from their CNS environment results in rapid and extensive downregulation of microglia-specific genes[146,147]; however, defined serum-free conditions have recently been developed that enable the investigation of signals required for microglia survival, development, and function[146].

Such approaches will need to be advanced while keeping in mind the multiple functional states of microglia and the high degree of plasticity of these cells. Given these variables, these functional states must be understood *in vitro* and tightly controlled, so that as biomarkers and therapeutics are being developed, they can be matched to specific functional states. Independent validation will be crucial so as to increase the likelihood of translating biomarkers and new therapeutics across species, or even from human iPSC-derived microglia, to the clinic.

Conclusions

We are just beginning to understand how microglia function in health and are altered in disease. Even so, our growing knowledge of the diverse roles of these cells in the healthy nervous system suggests that microglia-dependent CNS disorders may be reconceptualized as the result of aberrations in the physiological and homeostatic functions of microglia. Targeting these aberrant microglial functions, thereby returning homeostasis, may yield novel paradigms for therapies and biomarkers of microglia function and dysfunction for CNS disorders that were inconceivable under a neuron-centric view of the brain. Because recent information gained about the varying roles for microglia has come primarily from studies in the the mouse and in mouse models of disease, it is essential that we invest in developing new models of disease, including human iPSCs and organoids[144,146–150], to fully understand which findings translate from mouse to humans, and whether microglia-targeted therapeutic approaches being developed in mice can be used to treat human CNS diseases.

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The authors declare no competing financial interests.

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