

REVIEW

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The semantics of microglia activation: neuroinflammation, homeostasis, and stress

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Abstract

Microglia are emerging as critical regulators of neuronal function and behavior in nearly every area of neuroscience. Initial reports focused on classical immune functions of microglia in pathological contexts, however, immunological concepts from these studies have been applied to describe neuro-immune interactions in the absence of disease, injury, or infection. Indeed, terms such as ‘microglia activation’ or ‘neuroinflammation’ are used ubiquitously to describe changes in neuro-immune function in disparate contexts; particularly in stress research, where these terms prompt undue comparisons to pathological conditions. This creates a barrier for investigators new to neuro-immunology and ultimately hinders our understanding of stress effects on microglia. As more studies seek to understand the role of microglia in neurobiology and behavior, it is increasingly important to develop standard methods to study and define microglial phenotype and function. In this review, we summarize primary research on the role of microglia in pathological and physiological contexts. Further, we propose a framework to better describe changes in microglial phenotype and function in chronic stress. This approach will enable more precise characterization of microglia in different contexts, which should facilitate development of microglia-directed therapeutics in psychiatric and neurological disease.

Keywords: Microglia, Inflammation, Homeostasis, Stress, Parainflammation, Depression

Background

In the early 1900s, Pío del Río-Hortega described a new type of phagocytic brain cell, of mesodermal origin, that he termed “microglia” [1–3]. Decades later, microglia were recognized as macrophages, evidenced by morphological similarities to peripheral macrophages and their recognition by antisera against common monocyte markers [4–7]. Still, microglia are unique among macrophages. Unlike their fetal liver or bone marrow-derived relatives, microglia arise from embryonic yolk sack progenitors and maintain transcriptional and functional identities distinguishing them from other macrophages which may infiltrate the brain under pathological conditions [8–15]. As such, microglia are considered brain-resident

macrophages and are uniquely suited to regulate neural homeostasis and behavior. In particular, recent studies indicate that microglia can modulate neuronal activity, facilitate learning, and shape social behavior [16–24].

The underappreciated role of microglia in physiological conditions contrasts earlier work, which mainly focused on microglial function in pathological contexts such as injury and disease, where neuronal death or degeneration is observed [25, 26]. These initial studies characterized neuro-immune responses in such contexts and clearly defined the term “neuroinflammation,” which was guided by four primary features of peripheral inflammation: macrophage (microglial) activation, increased cytokines and chemokines, recruitment of peripheral immune cells, and local tissue damage [25, 27]. These studies also demonstrated that microglia and peripheral immune cells have dual roles: driving neuroinflammation and subsequent pathology, as well as resolving neuroinflammation

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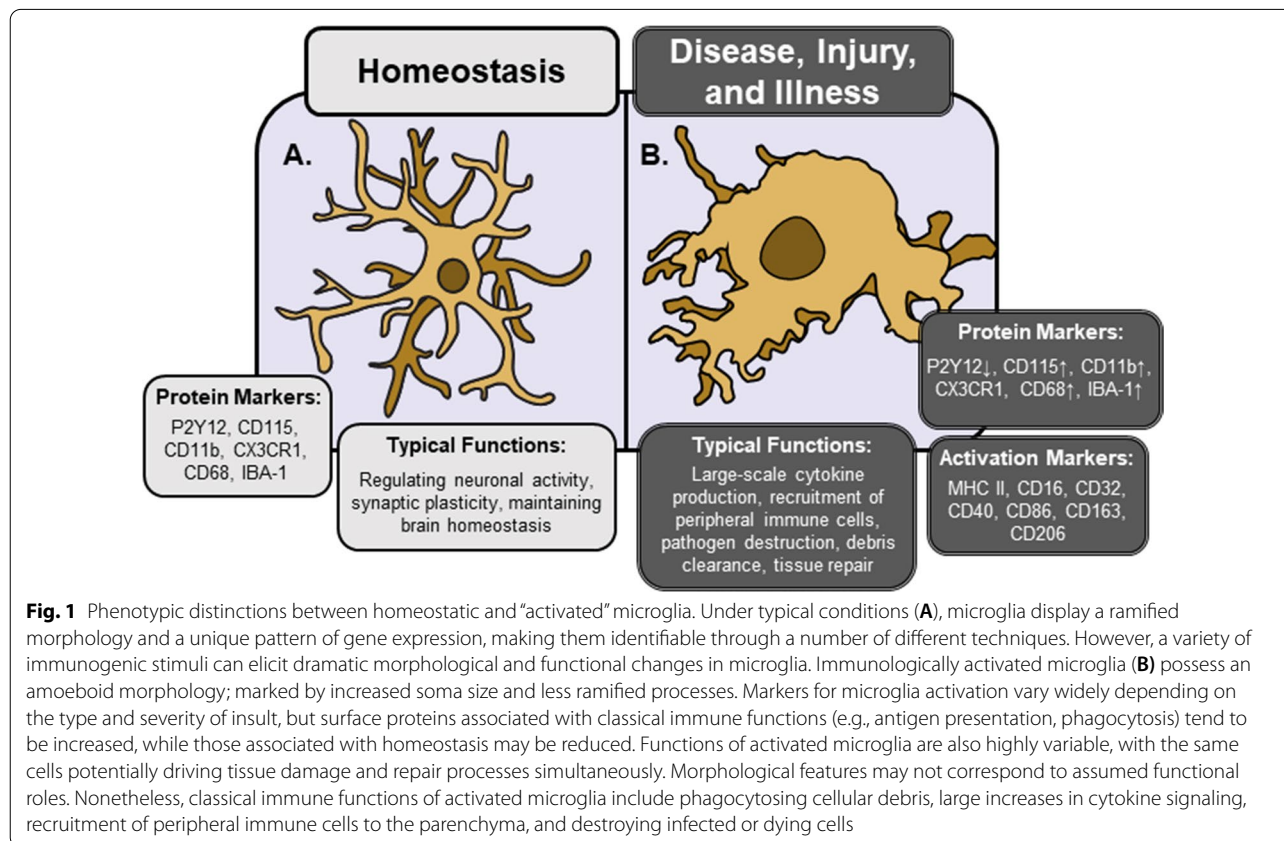


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and repairing the nervous system [28, 29]. Adding to the complexity of neuro-immune systems, these roles do not likely represent discrete functional states *in vivo*. Indeed, there is ample evidence that inflammation simultaneously drives tissue damage and repair [25, 30–33]. Nonetheless, these functions collectively represent various facets of microglia activation, which is classically identified by substantial increases in cytokine production, the adoption of an amoeboid morphology, and changes in relevant protein markers (Fig. 1) [34–37]. It should be noted that there is ongoing refinement of approaches to characterize macrophage/microglia activation [for a comprehensive overview, see: [25, 30, 32, 33]]. There are limitations to these definitions because biological systems do not fit cleanly into well-defined categories. As a result, terms such as “microglia activation” and “neuroinflammation” have broadened over the years to include most immune-related processes in the nervous system. While this has occurred in multiple areas of study, this review will focus on the functional states of microglia and their contributions to the neurobiology of stress.

Accumulating evidence indicates that microglia play a role in the synaptic and behavioral changes associated with chronic stress [38, 39]. However, there remain fundamental issues in the conceptual framework used to

integrate immunological concepts with stress research. Indeed, the language used to study peripheral macrophages in the context of infection is not adequate to describe microglial function in chronic stress; especially considering the vast biological differences between these two contexts. Despite this, researchers often use the term “neuroinflammation” to describe immune-related changes caused by chronic stress, which prompts comparisons to tissue injury or disease states and ultimately hinders our understanding of stress effects on microglia. This is significant because preclinical stress models are often used to gain insight into the neurobiology of psychiatric disorders. Thus, simplifying the role of microglia and neuro-immune systems in these models may lead to spurious therapeutic targets that ultimately hinder further clinical research. As advanced approaches (e.g., single cell RNA-Seq) improve our understanding of microglial phenotypes and functions, it is important to define specific features of microglia across model systems and disease states. This will increase the likelihood that congruent molecular and cellular pathways will be identified, which may facilitate the development of therapeutic approaches targeting microglia. In this review, we will discuss microglial functions in pathological and homeostatic conditions; as well as highlight the need to refine



terms used to describe microglia in different contexts. Moreover, we propose an alternative framework in which neuron–microglia interactions coordinate neurobiological adaptations to stress, congruent with an immune state called ‘parainflammation’ (not neuroinflammation).

Microglia in inflammatory contexts

Inflammation is triggered by immunogenic stimuli (i.e., pathogens or tissue injury), resulting in various biochemical cascades aimed at resolving these threats to homeostasis. Consequently, tissue damage and repair processes are enhanced during inflammation, often in tandem, until it resolves through complex regulatory mechanisms. In the brain, neuroinflammation manifests as elevated levels of pro-inflammatory cytokines, macrophage (microglia) activation, peripheral leukocyte infiltration, and damage to nervous tissue [25]. Although it is becoming increasingly clear that neuro-immune interactions play a role in most facets of brain function, it is typically under circumstances of disease, injury, and infection that all four of these hallmarks are present [27, 40]. In high enough concentrations (i.e., during neuroinflammation), immunogenic molecules can “activate” microglia, causing them to adopt an amoeboid morphology, and drastically increase their production of cytokines and reactive oxygen species (ROS). However these functional changes vary based on the type of stimuli and severity of insult [41]. Thus, in order to understand microglial function in a disease context, it is important to appreciate the intricacies of neuroinflammation. To this end, we will discuss a few examples of microglial function in neuroinflammatory contexts.

Multiple sclerosis and experimental autoimmune encephalomyelitis

Microglia activation is observed in various central nervous system (CNS) diseases and is important for coordinating the immune system’s resources during disease-associated neuroinflammation. For example, activated microglia are the main phagocytes observed in early stage multiple sclerosis (MS) lesions. This changes over time, with recruited bone marrow-derived macrophages taking over as the lesion advances [42, 43]. Further insights come from mouse hepatitis virus (MHV) infection, which causes demyelinating disease in models of experimental autoimmune encephalomyelitis (EAE). Early after MHV infection, microglia increase expression of cytokines which suppress viral infection and signal to adaptive immune cells [44, 45]. Ultimately, this contributes to T-cell and peripheral macrophage infiltration, increased brain cytokine levels, and tissue damage; here in the form of plaques or lesions where activated macrophages are believed to contribute to demyelination [45, 46]. Recent studies demonstrate that the initial microglial

response is a critical determinant of EAE severity. Depleting microglia with the colony stimulating factor 1 receptor (CSF1R) antagonist PLX5622 prior to MHV infection delayed virus clearance and was linked to reduced infiltration and activation of CD4⁺ T cells, as well as decreased production of interferon gamma (IFN- γ); a critical suppressor of viral replication [44, 47–49]. This dampened immune response was associated with an 80% increase in mortality [44, 47]. This is consistent with other studies reporting greater demyelination at peak disease and less remyelination during recovery in mice lacking microglia [47, 49, 50]. Thus, while microglia appear to play a role in cytokine production and demyelination in this model, it is evident that they also promote adaptive immune responses and aid in processes to repair tissue in EAE [45]. It remains to be seen whether microglial functions characterized in EAE precisely correspond to those occurring in patients with MS [43]. Nonetheless, this work highlights the complexities of neuroinflammation and microglial phenotypes in disease.

Ischemic stroke

Microglia are often first responders to brain or nerve injury. In fact, studies show that just minutes after ischemic stroke, microglia adopt an amoeboid morphology, display enhanced isolectin B4 binding, and have increased levels of complement receptor 3 (CR3, a.k.a. CD11b) as well as major histocompatibility complex (MHC) I and II [51]. These activated microglia produce high levels of TNF α , leading to endothelial necroptosis and BBB destruction [52]. This secondary tissue damage may also contribute to the infiltration of peripheral monocytes in the brain parenchyma, where they proliferate and gradually take over as the predominant phagocytes at the site of injury [53]. Both microglia and peripheral monocytes are involved with clearing debris after stroke, which attenuates inflammation; however they may also phagocytose injured yet viable neurons, leading to greater tissue damage [54–56]. While these findings indicate that microglia drive pathology in stroke, there is evidence to suggest microglia also play a neuroprotective role. Mice lacking TREM2, a receptor highly expressed by microglia and believed to play a role in phagocytosis, show exacerbated ischemic damage after stroke [57]. Moreover, injured cells release high quantities of purines (ATP/ADP), which microglia detect and extend their processes toward via the purinergic receptor P2Y₁₂ [58, 59]. Recent work showed that after stroke, neurons in the ischemic penumbra recruit microglial processes through a P2Y₁₂ dependent mechanism, leading to the formation of an immunological synapse between the two cells [17]. Notably, this was attenuated in P2Y₁₂ knockout mice and coincided with increased

neuronal calcium load, disrupted cortical network function, and greater area of ischemia-induced disconnection [17]. Altogether these findings show that microglia have dual roles in driving the pathological hallmarks of neuroinflammation and preserving and repairing damaged tissue in stroke [60].

Alzheimer's disease

Numerous studies demonstrate that microglia and neuroinflammation play a role in the pathogenesis of Alzheimer's disease (AD). Genome-wide association studies have suggested that individuals with variants of immune genes such as CD33 and TREM2 are at greater risk of developing AD, and single-cell RNA sequencing studies suggest that immunologically activated microglia are present in both animal models of AD and tissue from AD patients [61–65]. Additionally, studies in human tissue found increased trafficking of peripheral leukocytes in brain tissue from AD patients, suggesting blood–brain barrier (BBB) decline and immune-driven pathology in AD [66–68]. In line with this, 5 × and 3 × FAD mice, which overexpress human mutations associated with familial AD, show increased neutrophil extravasation into the parenchyma at sites of amyloid beta (A β) plaques, where they release extracellular traps and IL-17. Importantly, depleting neutrophils reduced A β ₄₂ levels and was associated with better cognitive outcomes, suggesting that neutrophils play a role in AD pathogenesis [66]. Collectively, these studies point to neuroinflammation as a contributing factor in AD, however the precise role of microglia in AD is more complicated [69]. As mentioned above, mutations affecting TREM2 function are risk factors for AD, and importantly, TREM2 is involved in A β clearance by microglia [70, 71]. Further, stimulating microglia proliferation via TREM2 agonism in mice engineered to express one of these human polymorphisms, led to decreased plaque load and neuronal dystrophy, suggesting that stimulating immune function from microglia may be beneficial in AD [72]. This is in line with recent work which identified a subtype of disease-associated microglia (DAM), which are associated with A β plaques and promote their clearance through a mechanism involving TREM2 [73]. Thus, rather than suppressing immune function altogether, it may be useful to promote selective microglia responses to help restore homeostasis [74]. In contrast, other work suggests that microglia release A β and tau “seeds”, which exacerbate AD progression [75, 76]. Given these complexities, additional research will be required to fully appreciate the role of microglia in AD.

Meningitis

In cases of CNS infection, neuroinflammation caused by microglia activation is critical for detecting and eliminating pathogens, but is not without consequence [77]. For instance, bacterial infection of the subarachnoid space causes astrocytes, endothelia, and microglia to secrete large amounts of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF- α), interleukin 1 beta (IL-1 β), and interleukin 6 (IL-6), which drive inflammation in the meninges [78]. These cytokines also activate endothelial integrins and stimulate chemokine secretion, promoting peripheral leukocyte recruitment [79, 80]. Further, *Streptococcal pneumonia*, the microorganism most commonly associated with bacterial meningitis, can penetrate the blood–brain barrier (BBB), and microglia react directly to the bacterium's cell wall in the parenchyma [79, 81]. In vitro models and rodent studies show that components of the pneumococcal cell wall cause microglia to produce nitric oxide, as well as a variety of different cytokines and chemokines, increasing leukocyte extravasation to the parenchyma [79, 82]. This leads to a predicament where on one hand, invading pathogens will trigger apoptosis via bacterial exotoxins, ultimately resulting in death [83, 84]. On the other hand, neuroinflammation caused by cytokines and ROS from the immune system can stop infection, but at the cost of associated tissue damage [78].

Peripheral infection

Exposure to pathogens in peripheral tissue elicits neuro-immune signaling and microglia responses that are drastically different from CNS infection. In preclinical models, viral (i.e., poly-IC) or bacterial components (i.e., lipopolysaccharide; LPS) are used to mimic peripheral infection and study associated neuro-immune interactions. This work shows that immune cells (often tissue-resident macrophages) in peripheral tissues initiate pro-inflammatory signaling cascades that affect other immune mediators. In particular, circulating cytokines promote IL-1 β secretion from macrophages in circumventricular organs, as well as perivascular macrophages and endothelia of the neurovasculature. These pro-inflammatory signals are transmitted to and propagated by microglia in the parenchyma. [85–87]. These microglia responses mediate important physiological (e.g., fever) and behavioral (e.g., lethargy) components of sickness that promote survival [88]. This form of microglial activation is transient and occurs without causing overt neuropathology [41, 88–92]. Recent reports indicate that peripheral administration of LPS can cause transient BBB disruption and microbleeds in the brain [90, 92]. These neuro-immune interactions may contribute to the observed changes in inflammatory mediators in

the brain, but further studies are needed to define these mechanisms. It is important to emphasize that peripheral infection alters microglia function indirectly, as viral (i.e., poly-IC) or bacterial components (i.e., lipopolysaccharide; LPS) do not typically reach the brain parenchyma, and that effects of LPS will vary depending on the route of administration, dose, and duration of exposure [93]. Thus, studies using immune challenges, such as LPS, need to consider how neuro-immune pathways (e.g., vagus nerve; endothelial cells) transmit immune signals to microglia and the brain, and whether peripheral inflammation in these models truly drives neuroinflammation as well.

Microglia in non-inflammatory contexts

Foundational to the idea of discrete macrophage activation states is the notion that these cells show limited activity or exist in a “resting” state prior to activation. Keeping with this, many studies note morphological shifts between the complex ramifications protruding from “resting” microglia in homeostasis and that of reactive or “amoeboid” microglia seen in diseased tissue [34, 35]. However, by focusing on microglia in the context of disease, these studies overlooked the dynamic nature of microglia in homeostasis. Seminal work utilized in vivo two-photon imaging in transgenic mice expressing EGFP driven by the *Cx3cr1* promoter to investigate microglial function under baseline conditions [94, 95]. These studies demonstrated that microglia display nearly constant movement, with their processes undergoing cycles of extension and retraction as they survey the extracellular space [94]. Further, the authors noted that neuronal activity appeared to influence process motility, and that many microglial processes contained pieces of phagocytized material which were gradually transported to the cell body. Thus, instead of quiescent cells in a “resting” state, this work demonstrated that microglia are constantly surveilling and utilize processes associated with immune activation (i.e., phagocytosis) in physiological conditions.

Further studies elaborated on this work and demonstrated that microglia are actively involved in neurodevelopment. In early development, neurons establish many more synaptic contacts than persist in a mature brain. As these young neurons send and receive signals to each other, synapses lacking sufficient input are gradually pruned while more active synapses are maintained [96, 97]. Interestingly, work by Stevens et al. showed that inactive projections from retinal ganglion cells (RGC) in the dorsal lateral geniculate nucleus (dLGN) were pruned in a complement-dependent manner [98]. In the periphery, the complement system comprised freely circulating proteins that, when bound to pathogens, trigger a signaling cascade which enhances the ability of macrophages to

detect and phagocytose those pathogens [99–102]. Later work by Schafer et al. demonstrated that microglia mediate engulfment of inactive RGC projections within the developing LGN in a complement- and activity-dependent manner, which allowed for typical circuit development [103].

A number of mechanisms have been shown to guide microglia-mediated synaptic pruning, with inhibition of these pathways linked to atypical behavioral phenotypes [22, 104–106]. For example, mice lacking either TREM2 or CX3CR1, both receptors highly expressed by microglia, show impaired synaptic pruning during development [107]. Consequently, mice lacking either of these receptors exhibit altered brain connectivity and deficits in social behavior in adulthood. Additional studies even show compromised LTP alongside deficits in contextual fear conditioning and spatial learning memory in CX3CR1-deficient mice. [24, 108, 109]. Expanding on these developmental studies, recent work suggests that the complement system regulates microglial phagocytosis of synapses in the adult hippocampus and contributes to forgetting [110]. Meanwhile other experiments indicate that microglial phagocytosis of the extracellular matrix around hippocampal neurons promotes memory consolidation via a mechanism dependent on neuron-derived IL-33. Collectively these findings indicate that microglial phagocytosis is not only triggered by pathogens or injury, but it is critical for proper neurodevelopment and neuroplasticity as well.

A similar theme emerges in work examining purine (e.g., ATP/ADP)-mediated chemotaxis by microglia. Accumulating evidence indicates that this process, traditionally associated with tissue damage (see previous section), guides homeostatic neuron-microglia interactions [111]. Different labs have consistently shown that stimulation of NMDA receptors on highly active neurons causes them to release ATP/ADP into the extracellular space, which is detected by microglial P2Y12 receptors [19, 105, 112–114]. Upon binding extracellular purines, G_i coupled P2Y12 receptors promote extension of microglial processes toward activated neurons [17, 18, 115, 116]. Studies examining neuron-microglia interactions in the visual cortex showed that visual experience caused microglia to interact with dendritic spines of V1 neurons, and that depriving animals of light during visual critical periods increased their phagocytosis of neuronal material [117]. This was driven in part by P2Y12 signaling, as disrupting P2Y12 during monocular deprivation limited microglial remodeling of V1 synapses, which in turn abrogated typical ocular dominance [105]. Interestingly, recent studies show that this process is opposed by noradrenergic signaling, as β_2 -adrenergic receptors on microglia were shown to limit experience-dependent

plasticity in awake mice by inhibiting microglial surveillance [118].

Beyond guiding neuroplasticity, convergence of microglia processes toward active neurons is implicated in regulating neuronal activity through physical contact. For example, microglia have been shown to rescue the somatic potential of hyperactive neurons by physically contacting their axons, while pharmacologically inhibiting microglial detection of neuronal activity (and thus limiting their contacts with neurons) is associated with increased neuronal death [18]. Similarly, neuronal hyperexcitability induced by kainic acid provokes more severe and more frequently lethal seizures in mice lacking P2Y₁₂ [19], further implicating microglia in regulating neuronal excitability. Perhaps the most direct evidence of this however comes from a recent study showing that upon detection, microglia catabolize extracellular ATP into adenosine, which in turn suppresses hyperactive neurons by binding to the adenosine receptor A₁R [119]. This inhibitory mechanism was crucial for attenuating seizure severity in mice treated with pro-convulsive doses of a D₁ agonist, demonstrating a protective role for microglia. Taken together these studies suggest ATP-mediated chemotaxis by microglia is not only important for responding to injury, but also for maintaining brain homeostasis by limiting pathological neural activity.

Another function of activated macrophages is secretion of growth factors and cytokines. This too has been observed in microglia absent neuroinflammation. For example, Parkhurst et al. showed that microglial brain-derived neurotrophic factor (BDNF) secretion was important for synaptic integrity. In these studies, microglia-specific depletion of BDNF reduced cortical expression of certain glutamate receptors, which subsequently altered NMDA and AMPA mEPSCs [20]. These deficits in synaptic function ultimately translated to reduced learning-associated spine growth and subsequent behavioral impairments in rotarod training, indicating that trophic support from microglia is an important neurobiological component of learning. Cytokines are also known to modulate synaptic plasticity under physiological conditions. Initial studies performed both in vivo and in acute hippocampal slices showed that *Il1 β* transcript was increased after LTP induction, and that application of IL-1 receptor antagonist (IL-1ra) interfered with LTP maintenance [120]. Similarly, mice engineered to overexpress IL-1ra in the brain show learning deficits, reduced GluA1/2 expression, and impaired BDNF signaling [121]. It should be noted that cytokine signaling is complex, and the same molecule might have entirely different effects depending on concentration, or the presence of other cytokines [122]. Consistent with this, IL-1 β has a concentration-dependent relationship with LTP. Under

physiological conditions, IL-1 β concentrations are relatively low and can enhance LTP and improve recall of a conditioned stimulus. In contrast, when IL-1 β concentrations are high (i.e., inflammation or aging), IL-1 β impairs LTP by acting at the synapse [123–125]. Other immune factors such as TNF α , IL-10, CXCL16, and CX3CL1 have also been implicated in LTP, with microglia acting either as potential sources of, or the cells affected by these molecules [126–131]. While much work is needed to fully appreciate microglial contributions to synaptic plasticity, these studies demonstrate the importance of neuro-immune interactions in homeostatic brain function.

Microglia in psychological stress: debating neuroinflammation

Clinical and preclinical studies conducted over the past two decades demonstrate that psychological stress influences various neuro-immune pathways [38, 132]. Early work revealed that both acute and chronic stressors increase levels of certain pro-inflammatory cytokines such as IL-1 β in the brain [133, 134]. Further studies specifically implicated microglia as the primary source of these cytokines, and showed that chronic stress altered microglial morphology [133, 135–138]. Collectively these changes were considered evidence of microglia activation, and therefore neuroinflammation, caused by psychological stress. However, as outlined above, neuroinflammation is characterized by macrophage activation, pro-inflammatory cytokine secretion, leukocyte recruitment, and tissue damage [25]. While there is good evidence that all four of these are present in conditions like MS, AD, stroke, and bacterial meningitis, it is not clear if the term neuroinflammation applies to stress-associated neuro-immune functions.

Recall that the effects of pro-inflammatory cytokines depend on other factors such as concentration [122]. During neuroinflammation, IL-1 β and TNF α concentrations can reach hundreds or even thousands of times higher than baseline levels, and promote cell death either by directly triggering apoptotic pathways in target cells, or by enhancing other neuroinflammatory processes associated with cellular damage [139–144]. While there is evidence that stress exposure influences IL-1 β and TNF α levels, the magnitude of this effect is considerably lower by comparison (<10 times baseline) and occurs in the absence of overt tissue pathology [41, 133, 135, 145, 146]. It should also be noted that outside the contexts of injury and disease IL-1 β and TNF α secretion in the brain facilitates synaptic plasticity, which is critical in maintaining homeostasis [124, 131, 147, 148]. As such, modest changes in pro-inflammatory cytokine expression may not indicate neuroinflammation and could represent an altogether different biological process.

A common finding reported in stress literature is the presence of “activated” microglia. As mentioned in the beginning of this review, the precise definition of macrophage activation is an area of ongoing debate and it is not clear what precisely constitutes an “activated”, “resting”, or “alternatively activated” macrophage or microglia [25, 30, 32, 33]. In Fig. 1, we provide some common features of microglia across immunogenic contexts, yet even these come with a multitude of caveats, as features of “activated” microglia in one context may not appear in another [34, 35, 37]. Further, as mentioned in the previous section, the idea of “activated” microglia presumes they are quiescent under typical conditions, which is not supported by recent data [149].

One of the common metrics to assess microglia activation are broad morphological shifts from small soma with ramified processes in homeostasis, to amoeboid with thicker and less complicated processes during neuroinflammation [36, 37]. Indeed initial reports indicated that stress caused microglia activation largely based on changes in morphological features [136]. However, evidence suggests that morphological changes in microglia do not define their functional state. Indeed microglia display dynamic and varied morphologies driven by local cues and these features may not overlap with assumed functions. For example, microglia in the developing subventricular zone and cortex display amoeboid morphology, yet the cytokines and growth factors they produce are essential for neuronal growth and differentiation [150, 151]. Even within the context of chronic stress, changes in microglial morphology vary between brain region, sex, species, stress paradigm, and stress duration, without specific biological or behavioral implications [136, 152–156]. In this context, it is not accurate to suggest that morphological changes in microglia alone can provide insight into their functional state. Moreover, it should be noted that these morphological analyses have been primarily reported in tissue immunolabeled with IBA-1, which does not fully reproduce microglial complexity. Indeed, studies using mice with a microglia-specific GFP reporter failed to observe such morphological changes after chronic social defeat stress, despite having more statistical power than previous reports [157]. These findings suggest that chronic stress exposure does not cause microglia activation in a way that resembles the immunological origins of the term [32, 33, 158].

Other studies have also reported that chronic stress promotes peripheral leukocyte trafficking in the brain, but this appears to be dependent on experimental approaches and context. In particular, studies using GFP⁺ bone marrow (BM)-chimeric mice showed that repeated social defeat (RSD) promotes signaling from microglia and caused infiltration of peripheral monocytes into

selective brain regions. These neuro-immune interactions were associated with reduced exploratory behavior in socially defeated mice [159–161]. However, further studies using LysM-GFP⁺, CCR2^{RFP}, and UBC^{GFP} reporter mice indicate that RSD promotes accumulation of peripheral myeloid cells (i.e., monocytes and granulocytes) in the perivascular space and choroid plexus; not in the brain parenchyma [157, 159, 162, 163]. These results suggest that development of the BM-chimera model enabled extravasation of myeloid cells into the brain following RSD [157, 159, 162–164]. Despite limited evidence for leukocyte trafficking in the brain, these studies did find that RSD caused a decline in BBB integrity [157, 165]. These stress-induced changes in the BBB were associated with vascular remodeling and the passage of signaling peptides, indicating that more subtle processes are involved during stress as compared to neuroinflammation [163, 165]. It is important to point out that accumulation of peripheral myeloid cells and loss of BBB integrity has been primarily observed in the RSD model. These findings are still significant, but it reinforces the fact that the context is critical; RSD is a unique paradigm as it involves aggressive encounters that can wound subordinate mice, potentially influencing their inflammatory profile [166–168]. Indeed, other stress models that use exposure to unstable or aversive environmental conditions (and lack physical confrontations or wounding) do not cause significant accumulation of peripheral macrophages in the brain [169].

In summary, while it is evident that stress engages neuro-immune pathways and alters microglia function, these effects are subtle and are not associated with immune-driven pathology. Despite these modest effects, neuro-immune interactions still play an important role in the behavioral and biological effects of chronic stress exposure. As such, a different framework is needed to accurately describe neuro-immune responses to stress.

Parainflammation: neuro-immune adaptations to homeostatic threats

Historically, neuro-immune interactions have been framed around existing immunological concepts derived from models of pathological conditions in which all four hallmarks of inflammation are present. By contrast, the behavioral and biological sequelae of chronic stress are best described as a consequence of prolonged adaptation to homeostatic threats, which, while associated with adverse effects, is not inherently pathological [170, 171]. For example, repeated stress exposure gradually attenuates hypothalamo–pituitary–adrenocortical (HPA) axis activation in response to a familiar challenge, ultimately reducing the physiological burden of chronic glucocorticoid secretion [170]. However, adaptation to chronic

stress also sensitizes HPA activation to new challenges, which, while appropriate in dangerous situations, can be disadvantageous in response to innocuous stimuli [170, 172]. Behavioral adaptations to chronic stress can be understood in a similar way. Recent work shows that social avoidance, the primary outcome of RSD in “susceptible” mice, is a learned behavior that does not generalize outside the aggressor’s strain and can be extinguished with repeated exposure in a safe environment [173]. In other words, “susceptible” mice learn to avoid an aggressor strain in a context that reliably predicts danger. Just as well, increased immobility in the forced swim test, a behavioral metric used in many other stress paradigms, might be an adaptive pro-survival behavior, as floating in an inescapable body of water conserves energy [174]. Nonetheless, these paradigms also alter behaviors such as sucrose preference, working memory, and effort-related choice [152, 175–179]. Thus, rather than overt pathophysiology (i.e., relating to disease or injury), chronic stress effects on brain and behavior can be framed as adaptations to adverse conditions, which can compromise typical function.

With this in mind, stress-induced functional changes in microglia resemble an intermediate tissue state termed parainflammation, in which stressed or malfunctioning cells engage tissue-resident macrophages to restore homeostasis [132, 180, 181]. In this conceptual framework (Fig. 2.), chronic psychological stress disrupts homeostatic brain function, provoking changes in microglia function. In coordination with other brain cells, microglia then facilitate neurobiological adaptations by initiating immune-related processes (i.e., cytokine release, phagocytosis). Of note, these microglia-mediated processes occur independent of other peripheral immune cells and at levels relative to physiological contexts. Ultimately, this may restore brain homeostasis, but may cause important behavioral and biological consequences.

In support of this, there is growing evidence that stress-induced changes in microglial function reflect coordinated interactions between microglia and neurons (and other cells in the brain) rather than dysfunctional immune processes. For example, chronic unpredictable stress (CUS) and glucocorticoids promote CSF1 signaling in the PFC, which elicits microglial phagocytosis of neuronal elements in the medial PFC [154, 169]. Ultimately,

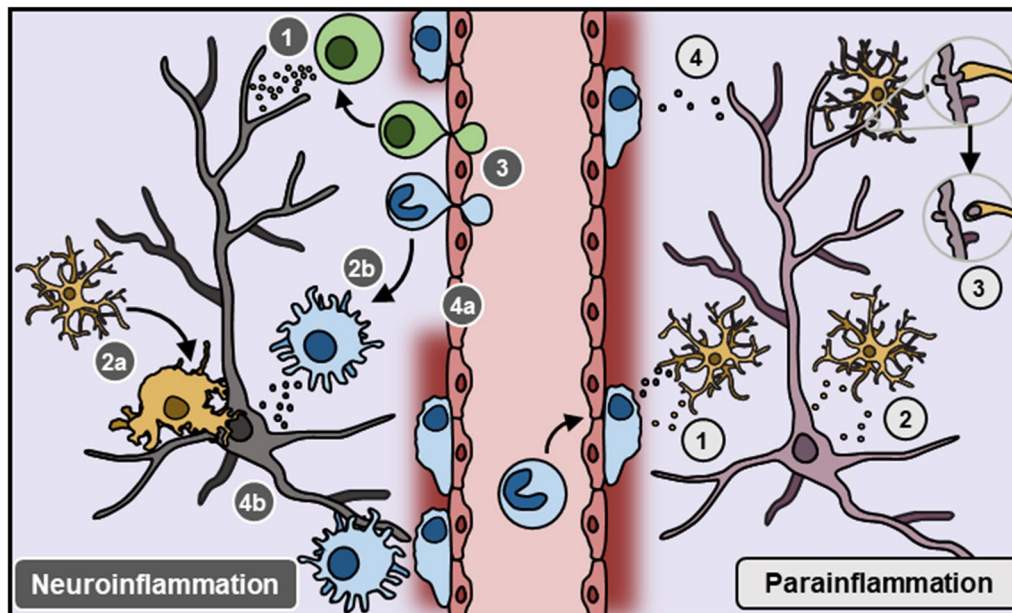


Fig. 2 Contrast between neuroinflammation and parainflammation. Neuroinflammation (left) describes immune-driven pathology which occurs in the course of disease, injury, or infection in the brain. This tissue state can be identified by four common molecular and cellular hallmarks illustrated on the left. These are: **(1)** high levels of pro-inflammatory cytokines, **(2a)** microglia and **(2b)** peripheral macrophage activation, **(3)** infiltration of peripheral leukocytes (e.g., bone-derived monocytes, T cells) to the parenchyma, and tissue damage such as **(4a)** BBB breakdown and **(4b)** neuron death. Neuro-immune systems can also be engaged by homeostatic challenges (i.e., psychological stress), leading to an intermediate tissue state termed parainflammation (right). While a formal definition of parainflammation is yet to be widely accepted in neuroscience, neuro-immune interactions previously reported in parainflammatory contexts are shown on the right. These include: **(1)** microglial recruitment of bone-derived monocytes to the perivascular space, **(2)** changes in cytokine signaling between neurons and microglia, **(3)** microglia-mediated neuronal remodeling, and **(4)** diffusion of small signaling peptides across the BBB

this neuronal remodeling by microglia contributes to synapse loss in the medial PFC and associated stress-induced behavioral changes [169, 182]. Interestingly, our recent work suggests that while synapse loss persists over the course of chronic stress, microglia-mediated neuronal remodeling diminishes over time, indicating that this is a coordinated process and that spine loss is maintained by other mechanisms [152].

Further support for the concept of parainflammation in stress research comes from emerging evidence that stress-induced changes in microglia correspond to stress-induced changes in neuronal activity. Previous work showed that repeated restraint stress increased microglial proliferation in the hippocampus; however, this was blocked by treatment with the glucocorticoid receptor antagonist RU486 or the NMDA receptor antagonist MK801 prior to restraint [183]. Other studies demonstrated that acute stress exposure increased c-Fos labeling in neurons within the periaqueductal gray (PAG), and that microglia adjacent to c-Fos positive neurons displayed morphological changes [184]. Furthermore, this was distinct from morphological changes caused by LPS treatment, as LPS altered microglial morphology in the PAG regardless of c-Fos labeling on nearby neurons, indicating that microglia respond to different stimuli in these contexts. Experiments conducted in our own lab also hint that neuronal activity drives microglial function in stress. We found that treatment with diazepam limited FosB staining in the medial PFC, which corresponded to attenuated stress effects on microglia-mediated neuronal remodeling, dendritic spine density, CSF1 signaling, and behavior [155]. This is in line with research in other model systems suggesting that CSF1 is released by hyperexcitable neurons, although this remains to be directly tested in the context of chronic stress [185, 186].

Because parainflammation is an adaptive process, it is important to consider these changes within the greater context of the stress model. Seminal work in stress neurobiology demonstrated that while acute stress drives glutamate signaling in the PFC and hippocampus of male animals, this reverses as stress exposure becomes chronic [179, 187–189]. It has previously been hypothesized that, while this change is not without consequences, it ultimately reflects a metabolic shift to meet the homeostatic challenges of stress [170, 171, 190–194].

In essence, glucocorticoids released during initial stress exposure cause long-lasting potentiation of AMPA and NMDA receptor currents in pyramidal neurons, leading to increased surface expression of these receptors and improvements in working memory [187–189, 195]. However, excess or prolonged glucocorticoid and glutamate stimulation can exacerbate excitotoxic insults by increasing intracellular Ca^{2+} , and high glucocorticoid

levels have profound effects on metabolism by depleting available energy and inhibiting cellular growth [170, 192, 193]. Thus, over time it may become beneficial to limit the signals associated with acute stress in these regions. Keeping with this, chronic stress is associated with decreased AMPA and NMDA receptor expression, blunted EPSCs in excitatory pyramidal neurons, and impairments in working memory [179, 196]. Previous reports are also largely consistent in documenting morphological changes such as dendritic retraction and loss of apical spines in pyramidal neurons after chronic stress exposure, which would make them less susceptible to the effects of excessive Ca^{2+} [197–201].

Given this context, the changes in microglial function arising from chronic stress are quintessentially parainflammatory processes. In this framework, acute stress exposure increases excitatory neurotransmission in a subset of brain regions [187, 188]. Microglia detect this shift in neuronal activity, and may modulate it through increased (but much lower than pathological levels) cytokine secretion [124, 135, 145, 202]. As stress exposure continues, activity-dependent signals from neurons, such as CSF1, increase and direct neighboring microglia to modulate neuronal activity through structural remodeling of neurons or release of neuromodulators [169]. These parainflammatory mechanisms are also accompanied by cell autonomous mechanisms in neurons, such as dendritic retraction and degradation of glutamate receptors, which maintain this neuronal phenotype [179, 197, 200, 203]. Collectively, these coordinated neuron–microglia interactions mitigate the effects of uncontrolled glutamate signaling and promote behavioral adaptations to adverse conditions. As an inadvertent result however, these changes may also promote longer-term behavioral and physiological consequences. Thus, while parainflammatory mechanisms are not associated with overt tissue pathology (as is the case in neuroinflammation), some aspects can be considered maladaptive.

Clinical implications

This is a semantic argument, but it is significant, as stress models are often used to inform our understanding of psychiatric conditions such as major depressive disorder (MDD). Indeed, preclinical work attributing stress effects to neuroinflammation and microglia activation are frequently cited by clinical studies claiming a similar etiology for MDD. These clinical reports indicate that some individuals with MDD have higher blood cytokine levels than controls, while others report increased PET detection of translocator protein (TSPO) during depressive episodes, which is interpreted as an indication of microglia activation and neuroinflammation [204, 205]. While

this work provides evidence of altered immune signaling in MDD, much like the basic research discussed in this review, these measures do not necessarily indicate that neuroinflammation or immune dysregulation underlies the etiology of MDD.

Indeed, mild infections or experimentally induced inflammation with LPS increase blood concentrations of inflammatory cytokines to much higher levels, suggesting that peripheral immune signaling in MDD differs from that observed during inflammation [206–208]. Similarly, although TSPO has been used as a proxy for microglia activation, the functional consequences of increased TSPO are not entirely understood [209]. In fact, PET imaging studies conducted in patients with MS show no difference in TSPO expression between activated macrophages in white matter lesions and those in normal appearing white matter or in healthy controls [210]. Other studies have indicated additional confounds to consider in TSPO studies, namely that TSPO tracers are bound by vascular endothelia and TSPO levels show maximal intensity after the resolution of inflammation [211, 212]. Further, preclinical studies indicate that TSPO expression is also increased in neurons, not microglia, after periods of heightened neurotransmission [213]. These findings suggest that the utility of TSPO in assessing microglia activation is limited by its lack of cellular and temporal specificity. The role of neuroinflammation in MDD was further challenged by recent clinical studies that showed microglia from individuals with MDD have increased expression of transcripts and proteins associated with homeostatic functions, rather than markers of inflammation [214].

Our focus on this literature is not intended to dismiss a potential relationship between MDD and inflammation outright. There is strong evidence to suggest that genuine inflammatory responses can disrupt mood in patients with chronic illness or comorbid inflammatory diseases [215–217]. In addition, recent findings demonstrate that there are differences in circulating immune factors and cells in subsets of MDD patients [218]. Considering the evidence however, it seems unlikely that MDD or the psychological effects of stress can be considered truly neuroinflammatory conditions on their own. And although neuro-immune interactions have clearly been implicated in MDD, they appear to reflect an intermediate immune state rather than classic inflammation. Our understanding of immune function in MDD is still developing and further research is needed to fully appreciate its complexity. As such, hypotheses that reduce multidimensional diagnoses like MDD to pathological immune function are not likely to advance treatments for patients. This necessitates the use of conceptual frameworks that integrate parainflammatory neuro-immune interactions

within a larger biopsychosocial context, as has been done in other areas of neuroscience and psychiatry [219–221].

Conclusion

As research on the involvement of neuro-immune systems in chronic stress expands, it is imperative that our models and conceptual frameworks be adapted to relevant data. Initial studies used existing disease-based terms to define stress-associated changes in microglial function. However, preclinical research demonstrates that neuro-immune responses to stress are distinct from CNS disease, injury, or infection and should not be characterized as neuroinflammation [25, 41]. The neurobiological changes that occur with chronic stress reflect adaptations required to meet the homeostatic demands of an unstable environment. We suggest that, before concluding a given neuro-immune interaction is evidence of neuroinflammation, investigators first consider whether the hallmarks of inflammation are also present. These include: substantially increased cytokine concentrations, macrophage activation, parenchymal infiltration of peripheral immune cells, and pathological tissue damage [25]. We also urge stress researchers to consider how observed neuro-immune changes compare to other model systems within and beyond that context. It is also worth considering if these changes are entirely detrimental, or if they might reflect an adaptive, or even beneficial (i.e., eustress) response to unstable conditions [222, 223].

Distinction between pathological (inflammation) and adaptive (parainflammation) functions of neuro-immune interactions are necessary if we hope to one day leverage them for therapeutic purposes. To this end, technologies such as single-cell RNA-Seq and time-of-flight mass cytometry can lend important insights, as these approaches enable researchers to understand the heterogeneity of cellular populations and can identify molecular patterns to distinguish various functional states [73, 224, 225]. This is particularly relevant to tissue-resident macrophages, such as microglia, as they display a remarkable degree of functional plasticity [31]. Given these technical advances and appreciation for the spectrum of microglia function, we propose that it is time to shift the conceptual framework used to describe neuro-immune function in non-disease contexts, such as stress. Indeed, there is substantial evidence that neuro-immune interactions in chronic stress resemble parainflammation; an intermediate immune state where immune responses are directed toward reestablishing homeostasis [181]. In the end, this framework will enable researchers to more accurately describe the functional role of microglia in various contexts; and we are hopeful this leads to new therapeutic strategies involving microglia.

Abbreviations

A1R: Adenosine A1 receptor; ADP: Adenosine diphosphate; AMPA: α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; A β : Amyloid beta; ATP: Adenosine triphosphate; BBB: Blood–brain barrier; BDNF: Brain-derived neurotrophic factor; BM: Bone marrow; CCR2: C–C Motif Chemokine Receptor 2; CNS: Central nervous system; CR3: Complement receptor 3; CSF1: Colony stimulating factor 1; CSF1R: Colony stimulating factor 1 receptor; CUS: Chronic unpredictable stress; CX3CL1: C-X3-C motif chemokine ligand 1; CX3CR1: C-X3-C motif chemokine receptor 1; CXCL16: C-X-C motif chemokine ligand 6; D1: Dopamine 1 receptor; dLGN: Dorsal lateral geniculate nucleus; EAE: Experimental autoimmune encephalomyelitis; EGFP: Enhanced green fluorescent protein; HPA: Hypothalamo–pituitary–adrenocortical; IFN- γ : Interferon gamma; IL-1 β : Interleukin 1 beta; IL-10: Interleukin 10; IL-1ra: IL-1 receptor antagonist; IL-6: Interleukin 6; IL-33: Interleukin 33; LPS: Lipopolysaccharide; LTP: Long-term potentiation; MDD: Major depressive disorder; mEPSC: Miniature excitatory postsynaptic current; MHC: Major histocompatibility complex; MHV: Mouse hepatitis virus; MS: Multiple sclerosis; NMDA: N-Methyl-D-aspartate; PAG: Periaqueductal gray; PET: Positron emission tomography; PFC: Prefrontal cortex Poly-IC: Polyinosinic:polycytidylic acid; P2Y12: Purinergic receptor P2Y, G-protein coupled, 12; RGC: Retinal ganglion cells; ROS: Reactive oxygen species; RSD: Repeated social defeat; TREM2: Triggering receptor expressed on myeloid cells 2; TSPO: Translocator protein; TNF- α : Tumor necrosis factor alpha; UBC: Ubiquitin C.

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Authors' contributions

ESW defined the topic of review. SCW and ESW prepared the manuscript. SCW created the figures. JLB aided in manuscript review and figure design. All authors read and approved the final manuscript.

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