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REVIEW ARTICLE

# Microglia communication: Parallels between aging and Alzheimer's disease

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## Abstract

Aging alters the functional integrity of the adult brain, driving cognitive impairments and susceptibility to neurodegenerative disorders in healthy individuals. In fact, aging remains the most dominant risk factor for Alzheimer's disease (AD). Recent findings have expanded our understanding of microglia function in the normal aging and AD brain, provoking an appreciation for microglia involvement in remodeling neuronal connections and maintaining brain integrity. This homeostatic function of microglia is achieved in part through the ability of microglia to interact extensively with and rapidly respond to changes in the brain microenvironment to enable adequate phenotypic transformations. Here, we discuss pro-inflammatory drivers of microglia transformation in aging and AD by focusing on the immune-modulatory functions of secreted factors, such as cytokines, complement factors and extracellular vesicles. We highlight the involvement of these secreted factors in aging and AD-associated cellular changes in microglia immune activation, surveillance function, and phagocytosis. Finally, we discuss how pro-inflammatory phenotypic changes associated with altered immune communication could both facilitate and exacerbate impairments in synaptic plasticity and cognitive function observed in the aged and AD brain.

## Introduction

Microglia are the resident macrophages of the brain that carry out important roles in maintaining brain homeostasis, such as pathogen recognition, phagocytic clearance and trophic factor release.[1–7] This homeostatic function is achieved in part through the ability of microglia to interact extensively with and rapidly respond to changes in the brain microenvironment.[8,9] Aging and neurodegenerative disorders, such as Alzheimer's disease (AD), are characterized by impaired homeostasis in the brain, and are accompanied by dramatic changes to the brain microenvironment and to the cellular characteristics of neuroglia.[10] Microglia are particularly sensitive to homeostatic perturbation in age and neurodegenerative disease, as evidenced by dramatic changes in morphology and function. Accumulating research suggests that these changes in microglia interfere with their supportive role in the brain, leading to synaptic dysfunction and consequent cognitive decline. In the present review, we discuss the drivers of microglia transformation in aging and AD by highlighting immune-modulatory functions of secreted factors, mainly cytokines, complement factors and extracellular vesicles (Fig. 1). The choice to focus on these mediators of cellular communication is influenced by the strong inflammatory status of aged and diseased brains, and the well-appreciated involvement of these factors in the regulation of inflammatory responses in peripheral immune cells. We highlight the involvement of these secreted factors in aging and AD-associated changes in microglia immune activation, surveillance function, and phagocytosis (Fig. 1). Furthermore, we discuss how phenotypic changes associated with altered immune communication could drive impairments in synaptic plasticity and cognitive function in the aged and AD brain (Fig. 1). Areas of similarity and divergence between aging and AD will be compared to gain

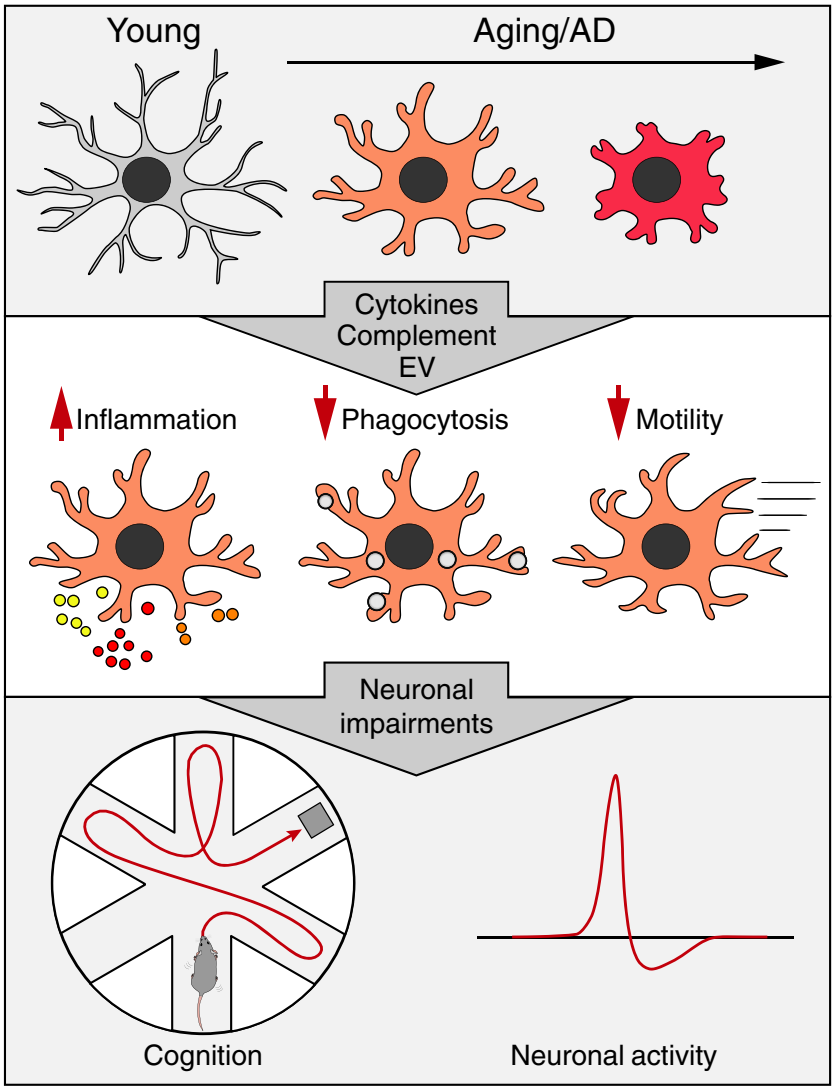


**Figure 1** Aging alters microglia communication driving age- and Alzheimer's disease (AD)-related functional impairments in the brain. Young microglia (gray) adopt a pro-inflammatory phenotype during aging (orange) and AD (red). These cellular changes alter microglia communication through cytokines, complements and extracellular vesicles (EV), promoting increased inflammation, decreased phagocytosis and decreased motility. Functional consequences of these aging- and AD-related changes in microglia communication result in neuronal impairments in synaptic plasticity and cognition.

insight into potentially convergent mechanisms of immune regulation between normal and diseased states in the aged brain.

## Microglia communication: Pro-inflammatory state in aging and AD

### Cytokines

Cytokines are a broad class of small proteins (5–20 kDa) that act as important mediators of cellular communication, both peripherally and in the central nervous system. In the brain, cytokines have been shown to regulate a variety of processes including cellular morphology, cell division, immune activation, migration and cell death.[4,10–12] Global pro-inflammatory brain cytokine levels increase with age, indicating a more inflamed status in the aged compared with young brain.[10] Given that microglia expression and production of inflammatory cytokines is much higher than other neuroglia, microglia are a likely culprit driving this age-related brain inflammation. Aged microglia are skewed toward a type 1 macrophage (M1) phenotype characterized by increased pro-inflammatory cytokine release, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and IL-6.[13,14] Mechanistically, decreased levels of the epigenetic repressor, SIRT1, during aging partially mediate the increased transcription of *IL-1*β in microglia.[15] Changes in the transcriptional regulation of *IL-1*β have been shown to alter IL-1β production promoting aging in microglia; however, whether such a phenomenon is recapitulated for other cytokines is unknown.[15]

In AD, the inflammatory profile of microglia is exacerbated with microglia expressing even higher transcript levels of *IL-1*β, *IL-12b* and *IL-23* under neurodegenerative disease conditions compared with age-matched normal controls.[16] Additionally, fibrillary or oligomeric amyloid beta (Aβ) induction of pro-inflammatory cytokine genes, 1Lβ, TNF-α, and IL-6, likely contributes to increased inflammation in AD brains.[17,18] Despite this observed augmentation of pro-inflammatory cytokine expression, microglia in aged and AD brains are not completely skewed to an M1 phenotype. Expression of anti-inflammatory mediators of alternative (M2) activation, IL-4 and IL-10, and their downstream effectors, chitinase-3 like 3 and arginase 1, are maintained at comparable levels in young and aged microglia.[19,20] Furthermore, bioinformatics analysis of gene expression profiles of microglia from aged and AD mice also shows that immune-related genes induced under these conditions are an intermediate mixture of M1 and M2 genes.[21] These findings raise important questions about the molecular mechanisms driving heterogeneity in microglia cytokine production in aging and AD. Local differences in immunoregulatory cues were recently reported to drive regional heterogeneity in microglia.[22] The extent to which cytokine signaling is involved in instructing microglia regional heterogeneity, and how these regulatory pathways become altered in aging and AD is yet to be investigated. Do microglia found in brain regions susceptible to age-related neurodegeneration, such as the hippocampus, produce more pro-inflammatory and fewer anti-inflammatory cytokines than other regions?

Microglia in aged and AD brains show a primed phenotype; in which the secondary response to insult is greatly exaggerated.[14,23] Little is known about cytokine signaling networks involved in maintaining this primed phenotype. It is also unclear what underlies the differential responses of primed aged microglia to pro- and anti-inflammatory cytokines.[11,24] These differences highlight the complexity of cytokine signaling in microglia and the need for in depth analysis of various aspects of cytokine signaling, such as regulatory, competitive and complementary pathways, to better understand aging- and AD-specific modulations.

## Complement factors

The complement system is critical for proper immune activation and response.[25,26] In the central nervous system, the complement system has also been implicated in non-immune functions, such as shaping neuronal connections.[27] Most brain cells produce complement proteins, although as immune cells, microglia are particularly well equipped to engage in complement signaling.[28] Microglia express most components of complement signaling including secreted factors (C1q, C3, C4), receptors (C1qR, C3R) and inhibitors (CD59).[28–30] The complement system appears to be involved in the brain's response to perturbation given that age, infection and disease result in strong induction of complement.[31] In both mice and humans, transcripts for various complement factors including *C1q*, *C3*, *C4*, *C3aR1* and *C5aR1* are elevated in forebrains and hippocampi during normal aging and AD conditions.[32–35] Induction of these genes is stronger in AD, suggesting additional AD-specific mechanisms for complement activation.[32] These differences between complement induction in aging and AD might partially result from the complement activating effects of Aβ and tau.[36–38] A recent genome wide association study (GWAS) found associations between variants of complement factors, *CR1* and *CLU*, and AD in human patients, thus providing further evidence for complement involvement in AD pathogenesis.[39] Direct evidence for the cellular mechanisms underlying these observed relationships between aging and AD-associated complement induction remains poorly understood. For instance, the role of microglia activation on the global upregulation of complement factors in aged and diseased brains remains largely unknown. Interestingly, a recent transcriptome analysis of young and aged microglia revealed age-associated changes in microglia genes that potentially affect complement signaling.[24] RNA levels for complement receptors, *C3ar1* and *C5ar1*, increase in aged microglia compared with young microglia, but the impact of these changes on complement factor binding and production in the aged brain is unknown.[24] Additional studies are required to clarify the effect of aging on the complement system at the protein level. A recent study identified significant increases in C1q protein levels in aged whole brain homogenates compared with young tissue.[40] Interestingly, C3 protein levels were maintained at similar levels in young and old brains – a finding that is contrary to previous RNA expression data showing increased *C3* expression with age.[32,40] This disparity might be due to differences in tissues used for the specific experimental paradigms. Alternatively, it is possible that C3 expression is regulated by additional post-transcriptional mechanisms in the aged brain. Future studies must provide insight into

regulatory networks driving changes in complement between young and aged microglia.

### Extracellular vesicles

Extracellular vesicles (EV) are membrane-bound vesicles that have emerged as mediators of intercellular communication in numerous cell types including neurons, astrocytes, oligodendrocytes, neural stem cells and microglia.[41] EV range in size from 30 nm to 1 μm, and are derived either from direct budding at the plasma membrane (microvesicles) or through endocytic maturation (exosomes).[41] EV transfer bioactive lipids, proteins, and RNA molecules that can alter cellular behavior under both physiological and pathological conditions. In the brain, EV have been studied most broadly for their involvement in various brain pathologies including glioma, Parkinson's disease, AD and prion disease.[42] From these studies, a role for EV in the spread of misfolding-prone proteins, such as Aβ, tau, α-synuclein and superoxide dismutase, has received considerable interest.[43] In AD, amyloid precursor protein (APP) cleavage products copurify with exosomes *in vitro,* and exosome proteins are found in association with Aβ plaques *in vivo*.[44] Furthermore, *in vivo* inhibition of exosomes dramatically reduces amyloid plaque deposition in a 5XFAD mouse model.[45] These findings suggest a role for EV in plaque deposition in AD; but much remains to be learned about the cellular mechanisms involved. Microglia represent an attractive cellular target for exosome-mediated modulation of AD pathology based on the strong phagocytic and secretory phenotypes of these cells. Microglia internalize exosomes purified from other neuroglia, including neurons, oligodendrocytes and astrocytes, as well as Aβ and tau-bearing exosomes.[46–48] Interestingly, microglia can redirect phagocytosed tau into the exosome pathway for secretion, thus directly linking microglia to tau spreading in AD.[48] Future studies are required to clarify these findings in other AD models, as well as to elucidate potential involvement of exosomes in driving microglia neuroinflammation. These questions can be greatly instructed by a more in-depth investigation of the roles of exosomes in microglia physiology. Exosomes have been implicated in regulating various cellular processes in peripheral immune cells, including proliferation, immune activation, antigen presentation and phagocytosis.[49,50] It is unclear whether or not any of these functions are similarly regulated by exosomes in microglia. Analysis of microglia-derived microvesicles and exosomes, however, showed the presence of various immune-modulatory molecules, such as IL-1β proprotein and its processing enzyme, major histocompatibility proteins, cathepsins, and integrins.[51,52] Furthermore, transfer of bioactive EV cargo can drive recipient cells into a phenotype similar to the exosome-producing cells.[53,54] Whether or not a similar phenomenon is observed in response to transfer of microglia-derived EV, and its potential impact on inflammation in the aged and diseased brain are intriguing questions in this nascent field.

## Microglia responses: Changes in cellular function in aging and AD

Along with the changes in intercellular communication discussed above, microglia in aging and AD brains undergo significant phenotypic changes characterized by morphological transformations and altered functionality. This section will explore the connections between aging- and AD-associated changes in microglia communication (cytokines, complement and EV release), and functional changes in microglia. Focus will be placed on aging- and AD-associated changes in microglia morphology, surveillance, and phagocytosis (Fig. 1).

### Morphology and activation

Microglia undergo dramatic changes in cellular morphology in response to perturbation of brain homeostasis as a result of injury, disease and infection.[55] Microglia adopt a more amoeboid morphology, characterized by larger cell bodies and shorter dendritic processes, in aged and AD brains compared to young microglia that show elaborate ramified processes and smaller cell bodies. These changes are observed in various regions of the aged brain including the retina, hippocampus and forebrain, as well as visual and auditory cortices, suggesting a global response of microglia to aging.[56–58] This age-related microglia transformation from ramified to amoeboid morphology is characteristic of microglia activation by pro-inflammatory signals. Stimulation of purified microglia cultures with interferon gamma, lipopolysaccharide, and TNF-α drives amoeboid transformation and production of pro-inflammatory cytokines known to increase with aging, including IL-1β, IL-6 and TNF-α.[59,60] In addition to cytokines, various lines of evidence support the involvement of complement proteins in promoting microglia activation, and thus morphological transformation. Stimulation with C1q induces microglia activation through WNT signaling, a pathway previously implicated in AD pathology.[61]

Ablation of C3 skews microglia towards an alternative (M2) activation phenotype, characterized by increased levels of IL-4 and IL-10 in the brain.[62] Additionally, complement signaling converges with other signaling pathways implicated in microglia activation during aging and AD, such as the Toll-like receptor pathway.[63,64] Protein deposits typical of AD, but not normally observed during aging, are also sufficient to drive changes in microglia activation and morphology. Fibrillary and oligomeric Aβ increase pro-inflammatory cytokine production in microglia *in vitro*.[65] In AD mouse models reactive microglia morphology is observed much earlier compared with wild-type controls, and plaque-associated microglia show more pronounced morphological transformation compared with microglia distant from plaques.[66,67] Unlike cytokines and complement, EV involvement in aging and AD neuroinflammation is less understood. While an indirect connection, the presence of pro-inflammatory signals, IL-1β proprotein, and tau tangles in microglia-derived EV suggest that these vesicles have the potential to drive age- and disease-related morphological changes in microglia.[48,51] Studies are still required to explore the capacity of EV cargo, including bioactive lipids and RNA molecules, to regulate microglia activation and morphological changes in the aged and AD brain.

### Surveillance

The morphological transformation of microglia in response to activating secreted factors underlies functional changes in these cells, including altered surveillance behavior. Microglia surveillance of the brain environment through constant dendritic process movement, and contact with the extracellular space and surrounding neuroglia is an important homeostatic function.[8] This surveying capability allows microglia to rapidly respond to external perturbations and to secreted factors. For example, microglia respond to injury- or death-induced increases in extracellular adenosine triphosphate (ATP) by moving dendritic processes toward site of perturbation and by altering branching complexity.[9] In the context of aging and AD, there is increasing evidence that this surveying function is impaired in microglia. Aged microglia have less extensive dendritic arborization, showing shorter and thicker processes with fewer branches compared with young microglia.[57,67] *In vitro* and *in vivo* imaging of microglia processes have also revealed marked differences in process dynamics between young and aged microglia.[57,68] Compared with young microglia, baseline process motility speed is reduced in aged microglia, and ATP-induced changes in process motility and dendritic area are blunted.[57,68] Interestingly, an age-dependent differential response to ATP stimulation was observed, such that young microglia responded by process extension, whereas aged microglia responded by process retraction. This finding raises questions as to the underlying mechanisms driving these age-associated differences in the regulation of microglia process dynamics. The purinergic receptors, P2 yr12 and P2 yr13, which are important regulators of microglia process motility, are decreased in aged microglia.[24] Similar to aged microglia, lipopolysaccharide-treated microglia also show process retraction in response to ATP, a phenomenon linked to both decreased P2 yr12 and increased adrenergic signaling.[69,70] Furthermore, microglia process extension toward an ATP gradient is ablated in P2Y12 receptor-deficient mice.[69,71] Together, changes in purinergic receptor expression also point to microglia process motility defects being mediated, at least in part, through microglia M1 activation. A direct analysis of purinergic receptor function in aged microglia is now necessary to provide insight into what causes the differential response of aged microglia to ATP. Furthermore, it is also unclear what upstream signals trigger aging-associated changes in purinergic receptor expression in microglia. Thus future experiments are required to identify specific secreted factors – including cytokines, complement and EV – directly regulating purinergic receptor-mediated microglia process dynamics in the brain during aging.

Consistent with reports on the aged brain, similar changes in microglia process dynamics are observed in the context of AD. For instance, in the APP/PS1 mouse model of AD, microglia process extension after laser microlesion was significantly delayed compared with age-matched wild-type controls.[72] Aβ pathology likely causes this disease-associated impairment in microglia process motility, at least in part. *In vivo*, plaque-associated microglia show more profound changes in process extension and retraction compared with microglia located distant from plaques.[73] Interestingly, it was recently proposed that microglia plaque association is a neuroprotective and adaptive behavior by microglia in AD brains to protect neighboring neurites from protofibrillar Aβ42 toxicity.[74] The authors, however, failed to discuss the mechanisms driving this adaptive microglia behavior. Are secreted factors necessary for this functional adaptation, if so, how do the secretory profiles of microglia proximal and distal to plaques differ?

## Phagocytosis

Microglia are the professional phagocytes of the central nervous system, tasked with recognizing and clearing extracellular debris, in order to maintain brain homeostasis under both physiological and pathological conditions. The phagolysosomal system, which is the terminal point for internalized debris, has long been appreciated to undergo aging-associated changes in microglia. Early electron microscopy studies identified cellular inclusions and condensed debris in the lysosomes of aged rat cortical microglia.[56] In addition, aged retinal and cortical microglia show accumulation of lipofuscin, a residual product formed as a result of oxidation of lysosome-associated lipoproteins.[14,75,76] These findings have led to the consensus that aging alters lysosome function in microglia. It stands to reason that these changes in phagolysosomal function in aged microglia might contribute to microglia aging phenotypes. In fact, microglia stimulation with A2E, a major constituent of ocular lipofuscin, alters microglia activation and complement expression.[77] In line with gene expression analysis of young and old retinal microglia, stimulation with A2E increases *CD68* while decreasing transforming growth factor beta expression.[24,77,78] A2E treatment of retinal microglia also altered the expression levels of complement regulatory proteins, complement factor b and complement factor h, but had minimal effects on the expression of pro-inflammatory cytokines, IL-1β and IL-6.[77] It remains unclear how well these findings are recapitulated in other brain regions, and what mechanisms underlie the differential induction of complement and cytokines after perturbation of microglia lysosomes. Furthermore, it is unknown how aging-associated lysosomal dysfunction alters exosome biogenesis. Does debris accumulation in aged microglia lysosomes favor the formation of secretory multivesicular bodies? These questions highlight the significance of the lysosome as a potential nexus for integrating extracellular and intracellular signals in microglia, particularly in the context of aging.

There is much less clarity about the effects of aging on the actual process of microglia phagocytosis. In peripheral macrophages, aging-associated decline in phagocytosis of invading bacteria and apoptotic cells has been described.[79] Aged microglia show some deficiencies in phagocytosis based on a recent report showing decreased bead and amyloid phagocytosis compared with young microglia.[80] These differences might be in part as a result of changes in microglia ability to recognize phagocytic targets. This is supported by a recent transcriptomic analysis showing age-dependent differences in the expression of receptors for environmental sensing, wherein aged microglia increase expression of genes for sensing microbial ligands, while decreasing genes for sensing endogenous ligands compared with young microglia.[24] Future studies are now required to corroborate the functional implications of these transcriptional changes in the microglia phagocytic process in the aged brain. Studies should investigate the impact of age-related changes in the recognition of endogenous ligands on microglia involvement in maintaining homeostasis in the aged brain.[10]

Pathological protein deposits, such as Aβ and tau in AD, place an additional burden on the phagocytic capacity of microglia. Microglia utilize a variety of receptors, such as triggering receptor expressed on myeloid cells (TREM2), Toll-like receptors and complement receptors, to either directly recognize or facilitate internalization of amyloid deposits.[10,81] Microglia can regulate plaque size *in vivo* presumably through phagocytosis, based on the lysosomal localization of internalized amyloid.[74,82] Complement involvement in regulating plaque deposition in AD has been studied extensively. C3 deficiency accelerates plaque deposition in a mouse model of AD, with more profound effects observed in older animals suggesting age-dependency for complement involvement.[62] In addition, the authors observed changes in IL-4 and IL-10 expression in C3 deficient mice compared with controls, suggesting complement regulation of other aspects of microglia communication, including cytokine production.[62] Future studies are required to fully define complement and cytokine regulation of microglia phagocytosis. The relationship between microglia and amyloid plaques is bidirectional in nature, with amyloid also exerting influences that alter microglia phagocytosis. Microglia isolated from AD forebrains show significant changes in genes represented by the GO term "lysosome", such as *CD68*, *Gusb* and *Cts1*.[16] Additionally, *ex vivo* studies identified impaired phagocytosis of bead microspheres by microglia derived from AD brains compared with age-matched healthy controls.[72] It should be noted, however, that it remains unclear if the observed changes in lysosome-related genes contribute to the phagocytic deficiency observed in microglia from AD brains. It is possible that the increased expression of lysosomal genes is a compensatory phenotype to allow microglia to deal with increased protein burden in AD. Investigations are now necessary to help clarify the etiology of these changes in microglia phagolysosomal system in

aging and AD, and the various regulatory mechanisms involved.

## Microglia actions: Implications for synaptic plasticity and cognition in aging and AD

Neuronal communication is contingent on synaptic networks that are established in an activity-dependent manner to facilitate cognitive functions, such as learning and memory.[83] During aging, reduced dendritic spine density and loss of synaptic plasticity are thought to drive cognitive impairments and susceptibility to age-related neurodegenerative diseases, including AD.[83,84] It is becoming more apparent that the numerous changes that occur in microglia communication and function in aging and AD have vital consequences on higher order brain processes. In this section, we describe the effects of microglial dysfunction on synaptic plasticity and cognitive function during aging and AD (Fig. 1).

### Synaptic plasticity

Microglia play a central role in establishing synaptic networks by remodeling synapses,[85,86] thereby making them important regulators of synaptic plasticity. Disruption of microglia function leads to synaptic deficits, including alterations in ocular dominance,[87] learning-dependent dendritic spine remodeling[6] and learning-dependent long-term synaptic strengthening – long-term potentiation (LTP). For instance, disruption of microglia activation using a DAP12 mutant mouse model (KΔ75) enhances hippocampal LTP.[88] Alternatively, the loss of the microglia-specific fractalkine receptor (Cx3cr1) leads to greater activation of microglia and subsequent reductions in LTP.[89] Interestingly, the reduction in LTP caused by Cx3cr1 ablation is rescued by inhibition of IL-1β signaling, arguing that downstream cytokine release regulates synaptic plasticity. Intriguingly, several pieces of evidence suggest that microglia-mediated inflammation, and the increase of cytokine levels, in aging lead to synaptic loss. For instance, in aging rats, pharmacological inhibition of microglia activation using minocycline lowers IL-1β release, while increasing LTP.[90] Additionally, aged neurons release less CD200, a microglia activation inhibitor, and suppression of microglia in aged mice with CD200fc improves LTP.[91] As microglia activation and IL-1β release cause LTP loss in young animals, these concordant results suggest that inflammatory release of cytokines plays a similar role in synaptic plasticity in young and aged brains. In the context of AD, inflammation is exacerbated and is also thought to negatively impact synaptic function.[92] Genetic manipulations targeting inflammation have actually proven beneficial for synaptic plasticity in AD models. For example, deletion of the NLRP3 inflammasome – which is upstream of IL-1β production – rescues LTP and reduces Aβ plaque load in an APP/PS1 mouse model of AD.[93] In humans, several GWAS reports have shown that mutations in microglia regulatory genes (triggering receptor expressed on myeloid cells[94,95] and CD33[96]) lead to sporadic AD; whereas an integrated bioinformatics approach identified disruptions in microglia-specific TYRO protein tyrosine kinase binding protein (TYROBP) signaling as the most strongly affected regulatory network in sporadic AD.[97] Interestingly, TREM/TYROBP signaling, in which CD33 is also involved,[98] is known to activate phagocytosis while suppressing Toll-like receptor-mediated inflammation.[99] Of great interest now is how disruption of these microglia networks in humans leads to downstream pathologies that disrupt synaptic plasticity. Given that microglia undergo prominent shifts towards inflammation and suppression of phagocytosis in aging and AD, it stands to reason that such shifts mediate in part the corresponding decline in synaptic plasticity observed with age.[80]

Developmental studies show that microglia play important roles in both elimination and maintenance of synapses. Recently, it has been posited that aging- and AD-related synaptic loss is regulated by microglia through increased complement signaling with age.[100] Genetic studies in mice have shown that deletion of C3 prevents age-associated synaptic loss and rescues LTP, arguing that increased complement signaling triggers synaptic loss during aging. In a human APP transgenic mouse model of AD (Tg2576), concomitant loss of C1q increases synaptic density.[101] Interestingly, oligomeric Aβ causes microglia to release increased amounts of C1q that mark synapses for elimination by microglia through the classical complement cascade.[102] Inhibition of C1q, C3 or loss of CR3 in an AD mouse model (J20) prevents early synaptic loss and LTP reductions driven by Aβ, further indicating that microglia mediate the synaptic dysfunction observed in AD.[102] Collectively, these findings show that microglia promote synaptic dysfunction observed during both aging and AD through complement signaling. Although oligomeric Aβ drives complement-mediated synaptic elimination by microglia in AD, corresponding drivers of complement activation pertinent to synapse loss during normal aging have yet to be identified. If

microglia utilize complement in equivalent roles for eliminating synapses in aging and AD, it is necessary for future investigations to elucidate whether convergent or divergent mechanisms of increased complement activation mediate aging-related versus AD-related synaptic changes. Along these lines, a recent and stimulating report has pointed to exosomes produced by neurons *in vitro* as capable of inducing both expression of several complement genes and synaptic pruning by microglia.[103] Given burgeoning studies that implicate exosomes in AD pathology, could it be that such secreted factors mediate complement activation and synaptic pruning in the aging brain?[44]

### Cognition: Learning and memory

Synaptic plasticity, LTP in particular, is thought to be the foundation of learning and memory.[83] As such, it follows that synaptic remodeling by microglia also modulates these higher order cognitive functions. Indeed, Cx3cr1 deficiency in mice results in greater microglia activation, diminished LTP and deficits in hippocampal-dependent learning.[89] Combining genetic loss of Cx3cr1 with AD mouse models also exacerbates cognitive impairments.[104] Although the constitutive loss of Cx3cr1 does not discount developmental effects, inducible ablation of microglia in adult mice also leads to deficits in learning and synaptic plasticity.[6] Cognitive deficits resulting from microglia ablation highlight the importance of properly functioning microglia for higher order processes in the adult brain. Correspondingly, changes in microglia communication and pro-inflammatory activation occurring in aging and AD might represent fundamental mechanisms driving associated cognitive decline. For example, macrophage-specific (including microglia) deletion of SIRT1 in mice, which leads to increased IL-1β production, results in impaired spatial learning and memory in both normal aging mice and in a neurodegenerative disease mouse model.[15] In a complementary study, genetic deletion of pro-inflammatory nuclear factor-κB signaling in the brain was capable of ameliorating impairments in hippocampal-dependent learning and memory in normally aging animals.[105] Impressively, manipulation of nuclear factor-κB signaling specifically in aged hypothalamic microglia was sufficient to promote cognitive restoration in the aged brain.[105] In the context of AD, genetically suppressing the inflammatory prostaglandin E2 signaling pathway specifically in microglia rescues memory deficits in an APP/PS1 mouse model of AD.[106] Inactivation of NLRP3, which conveys an anti-inflammatory (M2) phenotype on microglia, also improves hippocampal-dependent cognitive function, and increases phagocytosis of Aβ in an APP/PS1 mouse model of AD.[93] Additionally, inhibition of the complement cascade ameliorates age-related impairments in hippocampal dependent memory consolidation in normally aged mice, and rescues learning and memory function in AD models.[100] These findings not only implicate broad inflammation in age-related cognitive decline, but also specifically define a role for aged microglia. Although cytokine and complement signaling have similar effects on microglia-mediated regulation of synaptic plasticity and cognition, the cross-talk between these cellular mechanisms during aging and AD remains poorly understood, and is a topic that warrants further investigation.

## Conclusion

Microglia communication – by means of cytokine, complement and EV – elicits functional immune responses that become detrimental in aging and AD, leading to a non-permissive environment for proper neuronal activity. Gaining a better understanding of the changes in extracellular communication that lead to microglia dysfunction during aging and AD should take center stage in future studies. Insight into how microglia communication drives age- and AD-related neuronal impairments could prove vital in our quest to identify the means to prevent, or even restore, cognitive function in older adults by targeting neuroinflammation.

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## Conflict of interest

None declared.

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