Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Neurobiology

Microglia phagocytic mechanisms: Development informing disease

Rebecca M. Beiter[1], Patrick W. Sheehan[1] and Dorothy P. Schafer

Abstract

Microglia are tissue-resident macrophages and professional phagocytes of the central nervous system (CNS). In development, microglia-mediated phagocytosis is important for sculpting the cellular architecture. This includes the engulfment of dead/dying cells, pruning extranumerary synapses and axons, and phagocytosing fragments of myelin sheaths. Intriguingly, these developmental phagocytic mechanisms by which microglia sculpt the CNS are now appreciated as important for eliminating synapses, myelin, and proteins during neurodegeneration. Here, we discuss parallels between neurodevelopment and neurodegeneration, which highlights how development is informing disease. We further discuss recent advances and challenges towards therapeutically targeting these phagocytic pathways and how we can leverage development to overcome these challenges.

Addresses

Department of Neurobiology, Brudnick Neuropsychiatric Research Institute, University of Massachusetts Chan Medical School, Worcester, MA 01605, USA

Corresponding author: Schafer, Dorothy P. (dorothy.schafer@umassmed.edu)

(Schafer D.P.)

[1] These authors contributed equally to this work.

Current Opinion in Neurobiology 2024, **86**:102877

This review comes from a themed issue on **Neurobiology of Disease 2024**

Edited by **Eric Nestler** and **Erik Musiek**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.conb.2024.102877

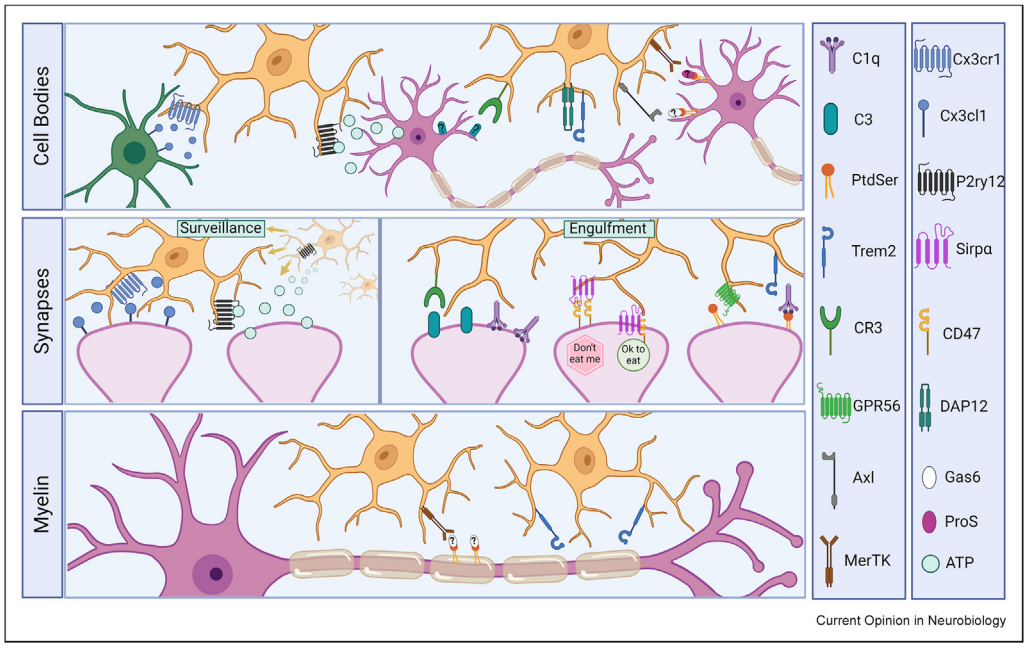
Introduction

Microglia are macrophages and the professional phagocytes of the central nervous system (CNS). As such, microglial phagocytosis of cellular and protein substrates is now appreciated to have a significant impact on multiple aspects of CNS development. This includes the engulfment of dead or dying neurons during normal programmed cell death, as well as the engulfment of synaptic and myelin membranes as these structures are sculpted into mature neural circuits. Intriguingly, in recent years, it appears that these developmental mechanisms are reactivated in neurological disease to drive or exacerbate loss of cells, synapses, and myelin. In addition, microglial phagocytic mechanisms are emerging as regulators of pathological protein aggregate accumulation, such as tau and amyloid β, during neurodegeneration. Here, we review the developmental mechanisms in which microglial phagocytosis of cellular substrates is now appreciated to be important, and we expand on how these mechanisms are informing neurological disease. While we recognize that there are numerous neurodevelopmental and neuropsychiatric disorders where microglial phagocytic mechanisms are relevant, this review will focus on these mechanisms in the context of neurodegenerative diseases (Alzheimer's disease, multiple sclerosis, etc.). We further appreciate the contribution of microglia to phagocytosis of protein aggregates, but we will focus on phagocytosis of cellular membranes in neurodegenerative disease contexts.

Microglial phagocytic mechanisms shaping the developing central nervous system

As the nervous system develops, new cells and synaptic connections are first generated in excess. Over time, some of these newborn cells and synapses are lost to allow for the remaining cells and synapses to strengthen and elaborate in the circuit. In the process, an abundance of cellular membranes must be cleared. Microglia are appreciated as major contributors to these clearance mechanisms (Figure 1). As a first step, microglia must be recruited to sites of dead or dying cells. Among these recruitment or 'find me' signals in the developing brain shown to regulate the clearance of dead and dying cells are the chemokine fractalkine (CX3CL1) signaling to its receptor CX3CR1 on microglia [1] and the microglial purinergic receptor P2RY12, presumably signaling through adenosine triphosphate (ATP) release from dead/dying cells [2]. After recruitment, cell surface phagocytic receptors on microglia mediate the clearance of these cell bodies, such as the Tyro3, Axl and MerTK(TAM) receptors MerTK and Axl [3], complement receptor 3 (CR3) [4,5], and DNAX activating protein 12 (DAP12) [6]. TAM receptors are a family of receptor tyrosine kinases that typically bind exposed

Figure 1



Receptors and ligands are known to play a role in the clearance of synapses, myelin, and cell bodies during development. Cell Bodies: Microglia phagocytose apoptotic cells during development and can even initiate apoptosis in otherwise viable cells. A number of different receptors have been shown to affect the developmental phagocytosis of cell bodies, such as TAM receptors, Trem2, and complement proteins. Synapses: Microglia are critical for pruning excess synapses in development. GPCR's Cx3cr1 and P2ry12 have been shown to play roles in attracting microglia towards synapses that will be engulfed. Other receptor-ligand pairs have been shown to directly mediate developmental synaptic pruning. Myelin: Myelin debris accumulates in the developing brain and is cleared by phagocytic microglia. Trem2 and TAM MertK have been shown to play roles in this process. Orange, purple, and green cells indicate microglia, neurons, and oligodendrocyte progenitor cells, respectively. Note that a question mark indicates that the role for Gas6, ProS, and C3 has not been clearly defined in the indicated contexts yet. PtdSer: phosphatidylserine; Trem2: triggering receptor expressed on myeloid cells 2; CR3: complement receptor 3; C3aR: complement C3a receptor 1; Axl: AXL receptor tyrosine kinase; MerTK: MER proto-oncogene, tyrosine kinase; Gas6: growth arrest-specific protein 6; ProS: protein S; GPR56: adhesion G protein-coupled receptor G1; ATP: adenosine triphosphate; Sirpα: signal-regulatory protein alpha; P2ry12: purinergic receptor P2Y, G-protein coupled 12; Cx3cl1: C-X3-C motif chemokine ligand 1; Cx3cr1: C-X3-C motif chemokine receptor 1; DAP12: DNAX activating protein 12 (DAP12).

phosphatidyl serine (PtdSer) to initiate phagocytosis, while DAP12 serves as an adaptor protein for triggering receptor expressed by myeloid cells 2 (TREM2) to bind PtdSer and regulate engulfment [7,8]. In contrast, CR3 is a complement cascade receptor that recognizes a variety of ligands but most notably binds a cleaved form of complement component C3, an opsin termed C3b [9]. Besides clearance of cell corpses, microglia can also play a role in initiating the cell death program prior to engulfment—a process termed "phagoptosis" (Reviewed in Ref. [10]). For example, early work showed that microglia engulf live neurons in the developing cerebellum, and when microglia are ablated, these neurons persist [11]. This same group showed that microglia are responsible for executing the death program through the release of superoxide during respiratory bursts. Very recently, it was shown that a subset of type I interferon (IFN-1)-responsive microglia exist in the developing cortex and are responsible for clearing whole neurons in the barrel cortex in response to whisker removal in neonate mice [12]. In IFN-1-deficient mice, there were excess neurons in the developing cortex, suggesting a role for microglial IFN-1 signaling in initiating the cell death program prior to the engulfment of neurons [12].

Similar to newborn cells, synaptic compartments also form in excess during development. In a process termed developmental synaptic pruning, a subset of these synapses must then be eliminated while the remaining synapses are maintained, strengthened, and elaborated. Microglia are known to regulate this synaptic pruning program in the developing brain through engulfment mechanisms. Like phagocytosis of dead/dying cells during development, molecules that modulate microglial proximity to synapses, including P2RY12 and CX3CR1, are involved [13–15]. An additional parallel to phagocytosis of dead/dying cells is complement-mediated synapse engulfment by microglia, in which the complement cascade components C1q and C3 bind synaptic membranes, followed by microglia-mediated engulfment via CR3 [16,17]. Since this initial work, it has now been demonstrated that complement

component C1q initially localizes to synapses upstream of their elimination by binding exposed phosphatidyl serine (PtdSer) on synaptic membranes, followed by microglial phagocytosis [18]. Additional molecules, including Trem2 and G protein-coupled receptor 56 (GPCR56), also bind PtdSer at synaptic membranes, leading to their elimination in the developing brain [19,20]. Suggesting a broader mechanism by which PtdSer modulates microglial engulfment function in development, exposure of PtdSer on myelin and subsequent engulfment of myelin via TAM receptors have also been observed in developing zebrafish [21].

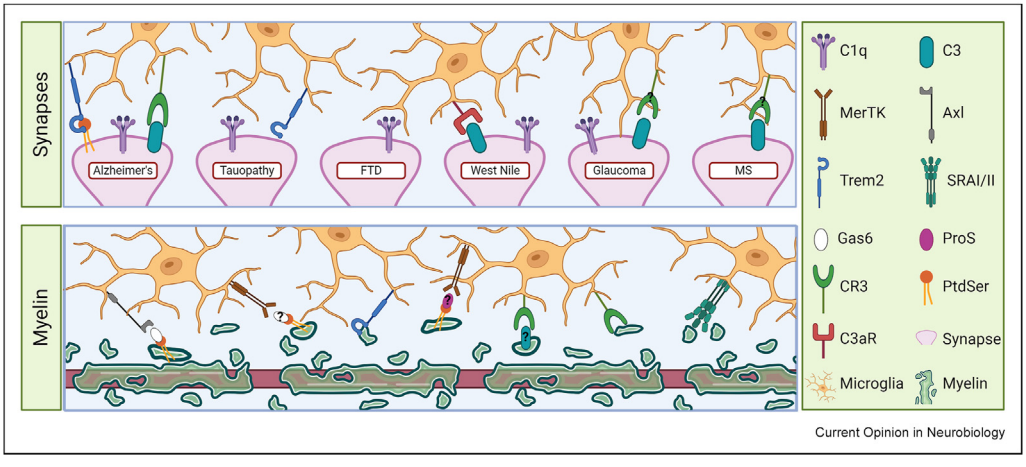
The data described above demonstrates positive regulators of phagocytosis. However, it is also important to consider the negative regulators of phagocytosis as a mechanism to limit the excess removal of cellular material by microglia in the developing brain. One example of this is through the "don't eat me" signal CD47 on neurons binding to its receptor, SIRPα, on microglia. Deletion of either CD47 or SIRPα leads to an upregulation of developmental synaptic pruning by microglia in the retinogeniculate circuit, the same circuit where complement has been implicated [22]. Interestingly, it appears that the cellular source of SIRPα is a key determinant of its ultimate effects on microglial pruning. That is, when CD47 binds microglial SIRPα, pruning of retinogeniculate synapses is inhibited in the lateral geniculate nucleus [22]. However, in the retina, if neuronal SIRPα binds CD47, this is permissive to retinal synapse pruning [23]. Additional work identified SRPX2 as a novel negative regulator of microglia-mediated synapse engulfment and pruning by binding C1q and preventing downstream complement activation and subsequent removal of developing synapses [24].

Out of all this work, it is clear that microglia are key regulators, shaping the cellular architecture of the developing brain through phagocytic mechanisms. When these mechanisms are inhibited, there are sustained increases in structurally intact cells, synapses, and myelin. Importantly, defects in these clearance functions can result in functional deficits that ultimately impact behavior. For example, loss of TREM2, CD47, and CX3CR1 has been shown to result in repetitive behavior and/or social deficits [19,25,26]. Most relevant to this review, this developmental work has laid the important mechanistic groundwork for understanding how microglial phagocytic mechanisms contribute to neurological disease, including neurodegenerative diseases. We expand on these parallels with neurodegenerative diseases in the following sections.

Developmental mechanisms reactivated to clear synapses and cell bodies during neurodegeneration

Similar to the developing brain, a large amount of neuronal material needs to be cleared during neurodegeneration (Figure 2). Also, many neurodegenerative disease risk genes regulate microglial phagocytosis [27].

Figure 2



Receptors and ligands are known to play a role in the clearance of synapses and myelin during disease. Synapses: Microglia have been shown to phagocytose synapses during multiple diseases, including Alzheimer's disease, tauopathies, frontotemporal dementia (FTD), West Nile viral infection, glaucoma, and multiple sclerosis (MS). In some cases, such as Alzheimer's disease and West Nile infection, both the receptor and ligand driving phagocytosis are known. In other cases, only the ligand driving synapse engulfment has been determined. Myelin: Microglia are important regulators of myelin debris clearance. Myelin clearance by microglia is a necessary step to facilitate remyelination and repair. A myriad of receptors have been shown to regulate this clearance, including Axl, MerTK (along with their obligate ligands Gas6 and ProS), Trem2, CR3, and SRAI/II. Note that a question mark indicates that the role for Gas6, ProS, and C3 has not been clearly defined in the indicated contexts yet. FTD: frontotemporal dementia; MS: multiple sclerosis; PtdSer: phosphatidylserine; Trem2: triggering receptor expressed on myeloid cells 2; CR3: complement receptor 3; C3aR: complement C3a receptor 1; Axl: AXL receptor tyrosine kinase; MerTK: MER proto-oncogene, tyrosine kinase; SRAI/II: scavenger receptor class A type I/II; Gas6: growth arrest-specific protein 6; ProS: protein S.

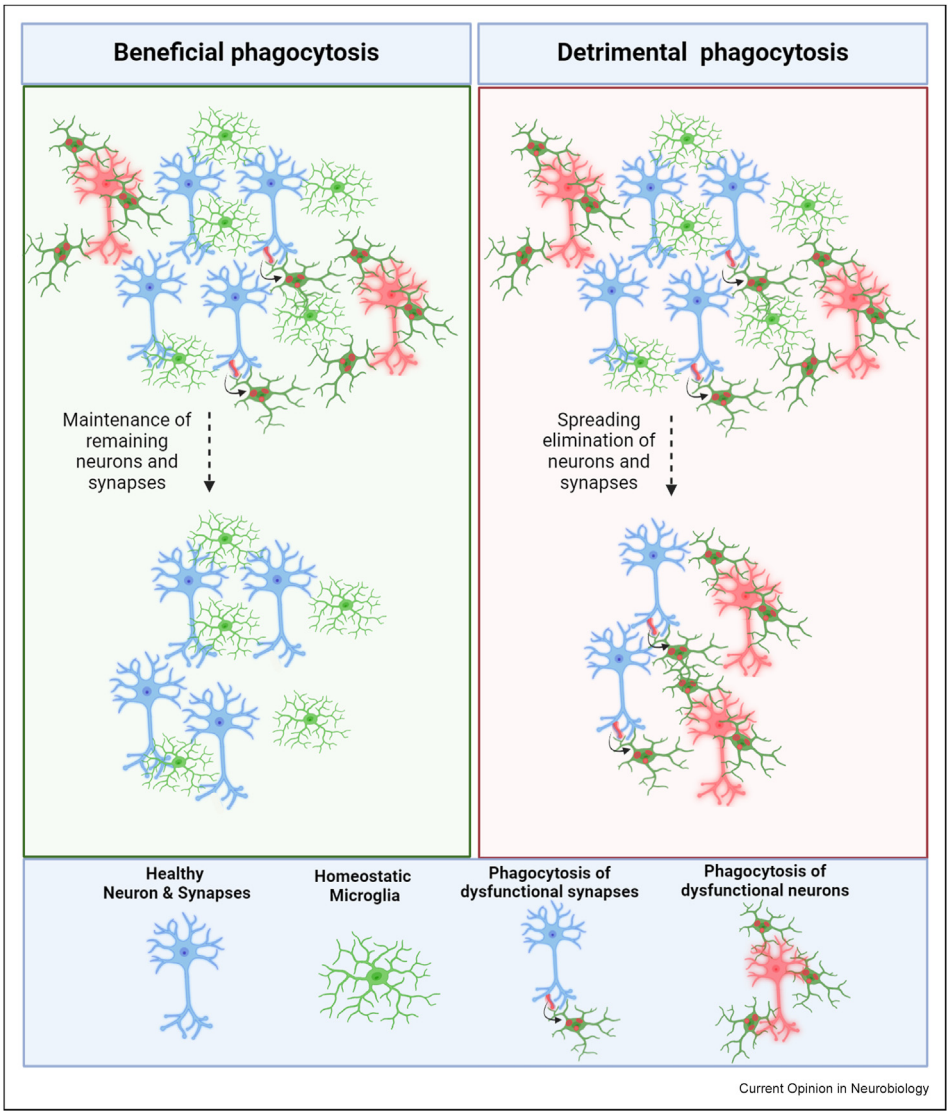
This has led to the hypothesis that the same developmental programs are reactivated to lead to phagocytic clearance by microglia in the context of neurological disease. Mechanisms of microglia-mediated clearance of synapses in neurodegenerative disease have been among the most widely studied and are discussed below.

Earlier work has demonstrated that complement proteins C1q and C3 mediate synapse loss in neurodegeneration in the context of glaucoma [17]. Like in development, these studies showed that complement proteins C1q and C3 localized to retinal synapses, and later work showed that this complement deposition leads to synapse elimination [17,28]. Another study showed that inhibiting C3 in the retina with an Adeno-Associated Virus(AAV)-based delivery approach was sufficient to protect against retinal ganglion cell death and axon degeneration [29], suggesting a more global mechanism by which complement leads to neurodegeneration in glaucoma. There has now been a flurry of studies demonstrating that complement-mediated phagocytic clearance of synaptic material by microglia contributes to neurodegeneration in aging [30], mouse models relevant to Alzheimer's disease (AD) [31–34], amyotrophic lateral sclerosis (ALS)/frontotemporal dementia (FTD) [35,36], West Nile virus (WNV) infection-induced neurodegeneration [37], Huntington's disease [38], and multiple sclerosis (MS) [39–42]. Importantly, inhibiting complement-mediated phagocytic signaling in these contexts preserves not only synapses but also circuit function and cognitive performance. In many cases, it appears that this mirrors the development whereby complement C1q and C3 are localizing to synapses, leading to their removal by phagocytic microglia. However, in other contexts, the mechanism by which complement regulates synapse engulfment differs (Figure 2). For example, in the Tau^{P301S} tauopathy mouse model, astrocytes, as well as microglia, engulf and eliminate synapses through a C1q-mediated mechanism [43]. In MS-relevant animal models, it was shown that complement C1q was not localized to retinogeniculate synapses [40]. Instead, complement C3 was at synapses, and C3 blockade was sufficient to prevent microglia-mediated synapse removal. In WNV-induced synapse loss, C1q and a cleavage product of C3 called C3d localize to hippocampal synapses, concomitant with microglia-mediated synapse engulfment via complement C3a receptor 1 or C3aR [37]. In addition, there are very recent studies that have identified factors upstream of complement components that function to regulate microglial phagocytosis during neurodegeneration. This includes recent work that showed the release of secreted phosphoprotein 1 (SPP1) by perivascular macrophages or fibroblasts drives microglial synapse engulfment in the APP^{NL-F} model of AD by inducing C1q deposition [44]. Another study identified neuronal pentraxin NPTX2 as a novel binding partner of C1q, which reduces C1q-mediated synapse removal by microglia in the Tau^{P301S} tauopathy mouse model [45]. Further, TREM2, which is a well-known genetic risk factor in AD, can induce a transcriptional program that induces complement gene expression [46,47]. Interestingly, Trem2 can also prevent C1q deposition by acting as a binding partner, thereby reducing microglial engulfment of synapses [48]. Other molecules that are upstream of microglial engulfment and possibly transcriptional regulation of complement are type I interferon, progranulin, TDP43, and C9orf72 [35,36,49–51]. Ablation of these genes enhances microglial engulfment of synaptic material, and the latter three molecules confer genetic risk for ALS and FTD.

The large number of accumulating studies demonstrates an intriguing connection between developmental microglia-mediated synapse pruning and synapse loss in disease. In many cases, complement proteins play a central role in directly regulating synapse loss by phagocytic microglia in disease. While less investigated in the context of neurodegeneration, 'phagoptosis' and/or the phagocytic clearance of dead or dying cells by microglia can also contribute to neurodegeneration. For example, microglia can regulate the cell death program in neurons in inflammatory conditions through Uridine 5' diphosphate(UDP)-dependent activation of the microglial purinergic receptor P2Y6 [52]. If P2Y6 is blocked, neurons remain intact for days after the insult. Intriguingly, many of these studies have suggested that this process of phaogoptosis promotes neurodegeneration. This disease-promoting role for microglial phagocytosis in neurodegeneration has also been supported in iPSC-derived human microglia, where the engulfment of apoptotic neurons, as well as other substrates, appears to drive a disease-associated microglial trajectory [53].

It is also important to consider the potential relationship between microglial phaogoptosis of stressed neurons and engulfment of synapses. In some cases, neurons may be dying first leading to downstream degeneration of neuronal processes and their synapses. In other cases, synapse loss may be happening first, such as in the case of complement-mediated synapse loss in multiple sclerosis discussed above [40]. Also, many studies investigating microglia-mediated neuronal and synapse engulfment in disease operate under the assumption that this is a detrimental process. However, there is growing evidence that engulfment of these substrates can have beneficial effects (Figure 3). Indeed, in models of excitotoxicity, microglia are involved in the phagoptosis of hyperactive cells to limit excitotoxicity through purinergic and CX3CL1 signaling [54,55]. Also, in an MS-relevant animal model, microglia and invading macrophages engulf components of dendritic spines that show elevated calcium activity [56]. Similarly, a recent study showed that exposure of hippocampal neurons to

Figure 3



Beneficial and detrimental roles for microglia-mediated phagocytosis of neurons and synapses. Left, in some cases, microglial removal of dysfunctional (e.g. hyperexcited) neurons or synapses is beneficial to protect surrounding neurons from, for example, excitotoxicity. Right, in other cases, this removal of dysfunctional neurons and synapses could render microglia in a state that promotes the removal of healthy neurons and synapses. This ultimately leads to the destruction of the entire circuit.

amyloid-beta (Aβ) *in vitro* and *in vivo* induces both synaptic hyperactivity, exposure of the "eat me" signal PtdSer, and microglial engulfment of these synapses via TREM2 [57]. This work is further supported by another study showing that dysfunctional TREM2 results in elevated synapse numbers and hyperactivity in mice [58]. Together, these data suggest that, in some cases, microglial phagoptosis of neurons or engulfment of synapses during neurodegeneration can be protective to eliminate hyperactive, dysfunctional cells and synapses. In parallel, it also seems that these processes can become exuberant in disease to promote neurodegeneration and subsequently cognitive decline (Figure 3).

Myelin degradation and clearance by microglia in neurodegenerative disease

Like synapses and cell bodies, myelin also goes through extensive remodeling in development and disease. In the disease context, microglial phagocytosis of myelin following a demyelinating insult has been shown to be beneficial for recovery by removing excess myelin debris to promote remyelination (reviewed in Ref. [59]). Just as with synapse engulfment, the mechanisms used to clear myelin debris and subsequently permit remyelination and repair parallel those used in development. This includes involvement of the complement system, whereby microglia upregulate complement components

in mouse models relevant to MS. While complement underlies synapse loss in this context [39,40], it can also play beneficial roles in tagging myelin debris for removal by microglia [60]. The latter process of debris removal is critical to promote remyelination [61]. Still, components of the complement cascade can also contribute to demyelination. For example, increased amounts of C3 or C5 cleavage production, C3a or C5a, result in more significant myelin loss in cuprizone-mediated demyelination, pointing to the role of these complement components in exacerbating demyelination [62,63]. Other developmental mechanisms that regulate myelin debris clearance during disease include MerTK and TREM2 [64,65]. For example, in the cuprizone model of demyelination, mice lacking TREM2 have more myelin debris present and decreased remyelination [65,66]. Antibody-mediated activation of TREM2 improves both myelin clearance and remyelination. Further investigation into this TREM2 mechanism revealed that TREM2 is involved in microglial processing of cholesterol from myelin debris, which is essential for creating an environment permissible for remyelination [67].

While previous work indicates that microglial clearance of myelin debris facilitates recovery and repair, recent new evidence has suggested this can also be unexpectedly detrimental. For example, one recent study showed that when an animal model of age-related myelin defects (*Cnp*$^{-/-}$;*Plp*$^{-/y}$ mice) was crossed to the 5xFAD AD-relevant mouse model, there was a higher plaque burden and reduced microglial clustering around amyloid plaques [68]. The authors further showed *in vitro* that when microglia phagocytose myelin, they are less able to engulf Aβ. This has led to an intriguing hypothesis that microglia preferentially clear myelin over other cellular substrates, and myelin phagocytosis may 'distract' microglia from clearing other proteins and cellular material. Additionally, studies indicate that the overburdening of microglia with myelin debris results in lipid droplet accumulation, elevated lipofuscin, increased proinflammatory cytokine production, increased reactive oxygen species, and defective phagocytosis [69–72]. This is particularly pronounced in lipid-droplet laden microglia, which are enriched in the white matter of the aged brain [69]. As developmental phagocytosis of synapses and myelin has also been observed, it may be interesting for future studies to consider preferential phagocytosis of substrates during development as well [16,18,20–24,73–75]. Taken together, data indicate that microglial phagocytosis may not always be beneficial, and continuous clearance of myelin debris, such as that seen in the aging brain, may result in microglial dysfunction. It will be important to further understand how the myelin sheath changes during aging-related neurodegenerative diseases, such as AD, and how this can contribute to dysfunction in microglial clearance mechanisms.

Summary and future directions

Microglial phagocytic mechanisms regulate many aspects of CNS development, from the clearance of excess synapses and cells to the sculpting of the developing myelin sheath. Strikingly, many of the same signaling mechanisms that regulate developmental phagocytosis of these cellular substrates appear to be involved in neurodegeneration to clear synapses, cell bodies, and myelin. While not the focus of this review, many of these phagocytic mechanisms also appear to play key roles in clearing toxic protein aggregates such as Aβ, tau, and α-synuclein (reviewed in Refs. [27,76]). Also, these mechanisms are likely involved in many other disease and injury contexts, including neurodevelopmental disorders and neuropsychiatric conditions.

An important consideration is the mechanism by which engulfment of cellular membranes by microglia occurs, which may not be mutually exclusive. In the case of synapses, this could be a result of phagocytosing an intact synapse, similar to how a microglia engulfs dead or dying cells. Alternatively, microglia could be weakening synapses through other mechanisms and clearing debris shed from a synapse. For example, studies have shown that molecules such as reactive oxygen species (ROS) secreted by microglia induce loss of dendritic spines in an AD-relevant mouse model [77]. Another possibility is trogocytosis, or the 'nibbling' of intact membranes, as suggested by recent live imaging studies in the developing Xenopus visual system and in mouse hippocampal slices [78,79]. This idea of engulfing intact membranes is further supported in the context of microglia-mediated myelin remodeling during development. By live-cell imaging in zebrafish, microglia were shown to directly consume myelin components from intact myelin sheaths versus engulfing shed myelin debris [75]. Also, microglia do not engulf substrates in a vacuum, and other cell types, such as astrocytes and Oligodendrocyte Precursor Cells(OPCs), are clearly important for either instructing microglia or clearing cellular substrates. While not discussed in this review, gaining a better understanding of this intercellular crosstalk in development will likely have important disease implications.

Another important consideration is that microglial phagocytosis can be either beneficial or detrimental, depending on the age, cellular or protein substrate, and disease context. On one hand, engulfment of synapses allows for the proper establishment and refinement of neural circuits in development, and engulfment of synapses and/or cell bodies in neurodegeneration may prevent hyperexcitability (Figure 3, left). Moreover, the removal of myelin accommodates the formation of new myelin sheaths in development and demyelinating disease. On the other hand, phagoptosis of cell bodies or synaptic engulfment by microglia could ultimately lead

to aberrant engulfment of nearby healthy neurons and synapses (Figure 3, right) and, ultimately, cognitive decline. Also, excessive myelin engulfment can cause impairment in long-term microglial phagocytic function. This is similarly the case when microglia phagocytose toxic protein aggregates, such as Aβ, tau, and α-synuclein. This can be beneficial to clear toxic proteins, but it can also be detrimental by inducing the release and spread of these protein aggregates throughout the CNS by microglia (reviewed in Ref. [80]). Thus, it remains a key open question whether therapies geared towards microglial phagocytic clearance mechanisms should target the enhancement or reduction of these phagocytic functions. Another complication for designing therapeutics is that a single pathway can regulate the clearance of different cellular substrates (e.g. myelin, synapses, etc.), but with opposing effects. Also, the timing may be key. For example, targeting clearance of dysfunctional synapses at early stages of disease may be beneficial, but this may become detrimental at later stages of disease when this becomes excessive and leads to the removal of otherwise healthy synapses. This becomes particularly important to disentangle when considering new disease modifying therapies. It also emphasizes the importance of developmental studies, which can then inform disease. In general, these developmental studies are faster and more amenable to genetic manipulation, with fewer factors that complicate the interpretation of outcomes. Developmental contexts are also conducive to invertebrate and zebrafish screens, which have largely informed the initial work on glia-mediated phagocytic clearance that is now appreciated to be important in disease [81–83]. Together, there is a high precedent for common microglial phagocytic mechanisms governing development and neurodegeneration. To move toward therapy, we must leverage development to inform disease but also better define how the timing and cellular substrate in disease dictate outcomes of phagocytic clearance by microglia.

Author Contributions

RMB, PWS and DPS wrote and edited the manuscript.

Declaration of competing interest

DPS is a consultant for Neurocrine Biosciences Inc., MindImmune Therapeutics Inc., and Switch Therapeutics, Inc.

Data availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by NIMH-R01MH113743 (DPS), NINDS-R01NS117533 (DPS), NIA-RF1AG068281 (DPS), the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (DPS), and NINDS-F32NS134683 (RMB).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Nemes-Baran AD, White DR, Desilva TM: **Fractalkine-dependent microglial pruning of viable Oligodendrocyte progenitor cells regulates myelination**. *Cell Rep* 2020, **32**, 108047.
2. Blume ZI, Lambert JM, Lovel AG, Mitchell DM: **Microglia in the developing retina couple phagocytosis with the progression of apoptosis via P2RY12 signaling**. *Dev Dynam* 2020, **249**: 723–740.
3. Fourgeaud L, Traves PG, Tufail Y, Leal-Bailey H, Lew ED, Burrola PG, Callaway P, Zagorska A, Rothlin CV, Nimmerjahn A, *et al.*: **TAM receptors regulate multiple features of microglial physiology**. *Nature* 2016, **532**:240–244.
4. Anderson SR, Roberts JM, Ghena N, Irvin EA, Schwakopf J, Cooperstein IB, Bosco A, Vetter ML: **Neuronal apoptosis drives remodeling states of microglia and shifts in survival pathway dependence**. *Elife* 2022, **11**, e76564.
5. Anderson SR, Zhang J, Steele MR, Romero CO, Kautzman AG, Schafer DP, Vetter ML: **Complement targets newborn retinal ganglion cells for phagocytic elimination by microglia**. *J Neurosci* 2019, **39**:2025–2040.
6. Wakselman S, Bechade C, Roumier A, Bernard D, Triller A, Bessis A: **Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor**. *J Neurosci* 2008, **28**:8138–8143.
7. Lemke G: **Phosphatidylserine is the signal for TAM receptors and their ligands**. *Trends Biochem Sci* 2017, **42**:738–748.
8. Zhong L, Zhang ZL, Li X, Liao C, Mou P, Wang T, Wang Z, Wang Z, Wei M, Xu H, *et al.*: **TREM2/DAP12 complex regulates inflammatory responses in microglia via the JNK signaling pathway**. *Front Aging Neurosci* 2017, **9**:204.
9. Jensen RK, Bajic G, Sen M, Springer TA, Vorup-Jensen T, Andersen GR: **Complement receptor 3 forms a compact high-affinity complex with iC3b**. *J Immunol* 2021, **206**:3032–3042.
10. Brown GC, Neher JJ: **Microglial phagocytosis of live neurons**. *Nat Rev Neurosci* 2014, **15**:209–216.
11. Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M: **Microglia promote the death of developing Purkinje cells**. *Neuron* 2004, **41**:535–547.
12. Escoubas CC, Dorman LC, Nguyen PT, Lagares-Linares C, Nakajo H, Anderson SR, Barron JJ, Wade SD, Cuevas B, Vainchtein ID, *et al.*: **Type-I-interferon-responsive microglia shape cortical development and behavior**. *Cell* 2024, **187**: 1–19.
13. Gunner G, Cheadle L, Johnson KM, Ayata P, Badimon A, Mondo E, Nagy MA, Liu L, Bemiller SM, Kim K-W, *et al.*: **Sensory lesioning induces microglial synapse elimination via ADAM10 and fractalkine signaling**. *Nat Neurosci* 2019, **22**: 1075–1088.
14. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, *et al.*: **Synaptic pruning by microglia is necessary for normal brain development**. *Science* 2011, **333**:1456–1458.
15. Sipe GO, Lowery RL, Tremblay ME, Kelly EA, Lamantia CE, Majewska AK: **Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex**. *Nat Commun* 2016, **7**, 10905.
16. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B: **Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner**. *Neuron* 2012, **74**:691–705.
17. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK,

Huberman AD, Stafford B, *et al.*: **The classical complement cascade mediates CNS synapse elimination**. *Cell* 2007, **131**: 1164–1178.

18. Scott-Hewitt N, Perrucci F, Morini R, Erreni M, Mahoney M, Witkowska A, Carey A, Faggiani E, Schuetz LT, Mason S, *et al.*: **Local externalization of phosphatidylserine mediates developmental synaptic pruning by microglia**. *EMBO J* 2020, **39**, e105380.

19. Filipello F, Morini R, Corradini I, Zerbi V, Canzi A, Michalski B, Erreni M, Markicevic M, Starvaggi-Cucuzza C, Otero K, *et al.*: **The microglial innate immune receptor TREM2 is required for synapse elimination and normal brain connectivity**. *Immunity* 2018, **48**:979–991 e978.

20. Li T, Chiou B, Gilman CK, Luo R, Koshi T, Yu D, Oak HC, Giera S, Johnson-Venkatesh E, Muthukumar AK, *et al.*: **A splicing isoform of GPR56 mediates microglial synaptic refinement via phosphatidylserine binding**. *EMBO J* 2020, **39**, e104136.

21. Djannatian M, Radha S, Weikert U, Safaiyan S, Wrede C, Deichsel C, Kislinger G, Rhomberg A, Ruhwedel T, Campbell DS, *et al.*: **Myelination generates aberrant ultrastructure that is resolved by microglia**. *JCB (J Cell Biol)* 2023, **222**.

22. Lehrman EK, Wilton DK, Litvina EY, Welsh CA, Chang ST, Frouin A, Walker AJ, Heller MD, Umemori H, Chen C, *et al.*: **CD47 protects synapses from excess microglia-mediated pruning during development**. *Neuron* 2018, **100**:120–134.e126.

23. Jiang D, Burger CA, Akhanov V, Liang JH, Mackin RD, Albrecht NE, Andrade P, Schafer DP, Samuel MA: **Neuronal signal-regulatory protein alpha drives microglial phagocytosis by limiting microglial interaction with CD47 in the retina**. *Immunity* 2022, **55**:2318–2335 e2317.

24. Cong Q, Soteros BM, Wollet M, Kim JH, Sia G-M: **The endogenous neuronal complement inhibitor SRPX2 protects against complement-mediated synapse elimination during development**. *Nat Neurosci* 2020, **23**:1067–1078.

25. Koshimizu H, Takao K, Matozaki T, Ohnishi H, Miyakawa T: **Comprehensive behavioral analysis of cluster of differentiation 47 knockout mice**. *PLoS One* 2014, **9**, e89584.

26. Zhan Y, Paolicelli RC, Sforazzini F, Weinhard L, Bolasco G, Pagani F, Vyssotski AL, Bifone A, Gozzi A, Ragozzino D, *et al.*: **Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior**. *Nat Neurosci* 2014, **17**:400–406.

27. Podleśny-Drabiniok A, Marcora E, Goate AM: **Microglial phagocytosis: a disease-associated process emerging from Alzheimer's disease genetics**. *Trends Neurosci* 2020, **43**: 965–979.

28. Williams PA, Tribble JR, Pepper KW, Cross SD, Morgan BP, Morgan JE, John SW, Howell GR: **Inhibition of the classical pathway of the complement cascade prevents early dendritic and synaptic degeneration in glaucoma**. *Mol Neurodegener* 2016, **11**:26.

29. Bosco A, Anderson SR, Breen KT, Romero CO, Steele MR, Chiodo VA, Boye SL, Hauswirth WW, Tomlinson S, Vetter ML: **Complement C3-targeted gene therapy restricts onset and progression of neurodegeneration in chronic mouse glaucoma**. *Mol Ther* 2018, **26**:2379–2396.

30. Shi Q, Colodner KJ, Matousek SB, Merry K, Hong S, Kenison JE, Frost JL, Le KX, Li S, Dodart JC, *et al.*: **Complement C3-deficient mice fail to display age-related hippocampal decline**. *J Neurosci* 2015, **35**:13029–13042.

31. Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, Merry KM, Shi Q, Rosenthal A, Barres BA, *et al.*: **Complement and microglia mediate early synapse loss in Alzheimer mouse models**. *Science* 2016, **352**:712–716.

32. Shi Q, Chowdhury S, Ma R, Le KX, Hong S, Caldarone BJ, Stevens B, Lemere CA: **Complement C3 deficiency protects against neurodegeneration in aged plaque-rich APP/PS1 mice**. *Sci Transl Med* 2017, **9**.

33. Dejanovic B, Huntley MA, De Maziere A, Meilandt WJ, Wu T, Srinivasan K, Jiang Z, Gandham V, Friedman BA, Ngu H, *et al.*: **Changes in the synaptic proteome in tauopathy and rescue of tau-induced synapse loss by C1q antibodies**. *Neuron* 2018, **100**:1322–1336 e1327.

34. Wu T, Dejanovic B, Gandham VD, Gogineni A, Edmonds R, Schauer S, Srinivasan K, Huntley MA, Wang Y, Wang TM, *et al.*: **Complement C3 is activated in human AD brain and is required for neurodegeneration in mouse models of amyloidosis and tauopathy**. *Cell Rep* 2019, **28**:2111–2123 e2116.

35. Lui H, Zhang J, Makinson SR, Cahill MK, Kelley KW, Huang HY, Shang Y, Oldham MC, Martens LH, Gao F, *et al.*: **Progranulin deficiency promotes circuit-specific synaptic pruning by microglia via complement activation**. *Cell* 2016, **165**:921–935.

36. Lall D, Lorenzini I, Mota TA, Bell S, Mahan TE, Ulrich JD, Davtyan H, Rexach JE, Muhammad AKMG, Shelest O, *et al.*: **C9orf72 deficiency promotes microglial-mediated synaptic loss in aging and amyloid accumulation**. *Neuron* 2021, **109**: 2275–2291.e2278.

37. Vasek MJ, Garber C, Dorsey D, Durrant DM, Bollman B, Soung A, Yu J, Perez-Torres C, Frouin A, Wilton DK, *et al.*: **A complement-microglial axis drives synapse loss during virus-induced memory impairment**. *Nature* 2016, **534**:538–543.

38. Wilton DK, Mastro K, Heller MD, Gergits FW, Willing CR, Fahey JB, Frouin A, Daggett A, Gu X, Kim YA, *et al.*: **Microglia and complement mediate early corticostriatal synapse loss and cognitive dysfunction in Huntington's disease**. *Nat Med* 2023, **29**:2866–2884.

39. Michailidou I, Willems JG, Kooi EJ, van Eden C, Gold SM, Geurts JJ, Baas F, Huitinga I, Ramaglia V: **Complement C1q-C3-associated synaptic changes in multiple sclerosis hippocampus**. *Ann Neurol* 2015, **77**:1007–1026.

40. Werneburg S, Jung J, Kunjamma RB, Ha SK, Luciano NJ, Willis CM, Gao G, Biscola NP, Havton LA, Crocker SJ, *et al.*: **Targeted complement inhibition at synapses prevents microglial synaptic engulfment and synapse loss in demyelinating disease**. *Immunity* 2020, **52**:167–182 e167.

41. Hammond JW, Bellizzi MJ, Ware C, Qiu WQ, Saminathan P, Li H, Luo S, Ma SA, Li Y, Gelbard HA: **Complement-dependent synapse loss and microgliosis in a mouse model of multiple sclerosis**. *Brain Behav Immun* 2020, **87**:739–750.

42. Absinta M, Maric D, Gharagozloo M, Garton T, Smith MD, Jin J, Fitzgerald KC, Song A, Liu P, Lin JP, *et al.*: **A lymphocyte-microglia-astrocyte axis in chronic active multiple sclerosis**. *Nature* 2021, **597**:709–714.

43. ** Dejanovic B, Wu T, Tsai MC, Graykowski D, Gandham VD, Rose CM, Bakalarski CE, Ngu H, Wang Y, Pandey S, *et al.*: **Complement C1q-dependent excitatory and inhibitory synapse elimination by astrocytes and microglia in Alzheimer's disease mouse models**. *Nat Aging* 2022, **2**:837–850.
In the Tau^{P301S} model, the authors show that both astrocytes engulf excitatory synapses while microglia engulf inhibitory synapses via C1q. They further show in a model with amyloid and tau pathology that loss of Trem2 impaired microglial phagocytosis of inhibitory synapses and an increase in astrocyte-mediated phagocytosis of synapses.

44. * De Schepper S, Ge JZ, Crowley G, Ferreira LSS, Garceau D, Toomey CE, Sokolova D, Rueda-Carrasco J, Shin S-H, Kim J-S, *et al.*: **Perivascular cells induce microglial phagocytic states and synaptic engulfment via SPP1 in mouse models of Alzheimer's disease**. *Nat Neurosci* 2023.
The authors showed that perivascular cells can induce microglia to engulf synaptic material in an Alzheimer's disease-relevant model via the release of SPP1.

45. * Zhou J, Wade SD, Graykowski D, Xiao M-F, Zhao B, Giannini LAA, Hanson JE, van Swieten JC, Sheng M, Worley PF, et al.: The neuronal pentraxin Nptx2 regulates complement activity and restrains microglia-mediated synapse loss in neurodegeneration. Sci Transl Med 15:eadf0141.The study showed that Nptx2 is a novel binding partner for C1q that serves to normally restrict C1q function and microglia-mediated synapse loss in TauP301S mice.

46. Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, Beckers L, O'Loughlin E, Xu Y, Fanek Z, *et al.*: **The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases**. *Immunity* 2017, **47**:566–581 e569.

47. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, *et al.*: **A unique microglia type associated with restricting development of Alzheimer's disease**. *Cell* 2017, **169**:1276–1290 e1217.

48. * Zhong L, Sheng X, Wang W, Li Y, Zhuo R, Wang K, Zhang L, Hu D-D, Hong Y, Chen L, *et al.*: **TREM2 receptor protects against complement-mediated synaptic loss by binding to complement C1q during neurodegeneration**. *Immunity* 2023, **56**:1794–1808.e1798.
Both TREM2 and C1q have been found to regulate synaptic phagocytosis independently. This study demonstrates that these two proteins interact to prevent synapse engulfment.

49. Paolicelli RC, Jawaid A, Henstridge CM, Valeri A, Merlini M, Robinson JL, Lee EB, Rose J, Appel S, Lee VM, *et al.*: **TDP-43 depletion in microglia promotes amyloid clearance but also induces synapse loss**. *Neuron* 2017, **95**:297–308 e296.

50. Roy ER, Wang B, Wan YW, Chiu G, Cole A, Yin Z, Propson NE, Xu Y, Jankowsky JL, Liu Z, *et al.*: **Type I interferon response drives neuroinflammation and synapse loss in Alzheimer disease**. *J Clin Invest* 2020, **130**:1912–1930.

51. Roy ER, Chiu G, Li S, Propson NE, Kanchi R, Wang B, Coarfa C, Zheng H, Cao W: **Concerted type I interferon signaling in microglia and neural cells promotes memory impairment associated with amyloid beta plaques**. *Immunity* 2022, **55**:879–894 e876.

52. Neher JJ, Neniskyte U, Hornik T, Brown GC: **Inhibition of UDP/P2Y6 purinergic signaling prevents phagocytosis of viable neurons by activated microglia in vitro and in vivo**. *Glia* 2014, **62**:1463–1475.

53. * Dolan MJ, Therrien M, Jereb S, Kamath T, Gazestani V, Atkeson T, Marsh SE, Goeva A, Lojek NM, Murphy S, *et al.*: **Exposure of iPSC-derived human microglia to brain substrates enables the generation and manipulation of diverse transcriptional states in vitro**. *Nat Immunol* 2023, **24**:1382–1390.
This author fed amyloid beta, synaptosomes, apoptotic neurons, or myelin debris to human iPSC-induced microglia and showed that this induced transcriptional signatures *in vitro* that mirrored *in vivo* transcriptional states that resembled disease-associated microglia (DAMs).

54. Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K, Shinozaki Y, Ohsawa K, Tsuda M, Joshi BV, Jacobson KA, Kohsaka S, Inoue K: **UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis**. *Nature* 2007, **446**:1091–1095.

55. Noda M, Doi Y, Liang J, Kawanokuchi J, Sonobe Y, Takeuchi H, Mizuno T, Suzumura A: **Fractalkine attenuates excito-neurotoxicity via microglial clearance of damaged neurons and antioxidant enzyme heme oxygenase-1 expression**. *J Biol Chem* 2011, **286**:2308–2319.

56. Jafari M, Schumacher AM, Snaidero N, Ullrich Gavilanes EM, Neziraj T, Kocsis-Jutka V, Engels D, Jurgens T, Wagner I, Weidinger JDF, *et al.*: **Phagocyte-mediated synapse removal in cortical neuroinflammation is promoted by local calcium accumulation**. *Nat Neurosci* 2021.

57. ** Rueda-Carrasco J, Sokolova D, Lee SE, Childs T, Jurčáková N, Crowley G, De Schepper S, Ge JZ, Lachica JI, Toomey CE, *et al.*: **Microglia-synapse engulfment via PtdSer-TREM2 ameliorates neuronal hyperactivity in Alzheimer's disease models**. *EMBO J* 2023.
This study demonstrated that exposure to amyloid beta induces hyperexcitability in synapses, which then results in PtdSer exposure and microglial engulfment of synapses via TREM2. The authors hypothesize that microglial engulfment of synapses in Alzheimer's disease may be protective by limiting hyperexcitability.

58. Das M, Mao W, Voskobiynyk Y, Necula D, Lew I, Petersen C, Zahn A, Yu GQ, Yu X, Smith N, *et al.*: **Alzheimer risk-increasing TREM2 variant causes aberrant cortical synapse density and promotes network hyperexcitability in mouse models**. *Neurobiol Dis* 2023, **186**, 106263.

59. Kent SA, Miron VE: **Microglia regulation of central nervous system myelin health and regeneration**. *Nat Rev Immunol* 2023.

60. Rotshenker S: **Microglia and macrophage activation and the regulation of complement-receptor-3 (CR3/MAC-1)-mediated myelin phagocytosis in injury and disease**. *J Mol Neurosci* 2003, **21**:65–72.

61. Lloyd AF, Davies CL, Miron VE: **Microglia: origins, homeostasis, and roles in myelin repair**. *Curr Opin Neurobiol* 2017, **47**:113–120.

62. Ingersoll SA, Martin CB, Barnum SR, Martin BK: **CNS-specific expression of C3a and C5a exacerbate demyelination severity in the cuprizone model**. *Mol Immunol* 2010, **48**:219–230.

63. Saez-Calveras N, Stuve O: **The role of the complement system in Multiple Sclerosis: a review**. *Front Immunol* 2022, **13**, 970486.

64. Shen K, Reichelt M, Kyauk RV, Ngu H, Shen Y-AA, Foreman O, Modrusan Z, Friedman BA, Sheng M, Yuen TJ: **Multiple sclerosis risk gene Mertk is required for microglial activation and subsequent remyelination**. *Cell Rep* 2021, **34**, 108835.

65. Cignarella F, Filipello F, Bollman B, Cantoni C, Locca A, Mikesell R, Manis M, Ibrahim A, Deng L, Benitez BA, *et al.*: **TREM2 activation on microglia promotes myelin debris clearance and remyelination in a model of multiple sclerosis**. *Acta Neuropathol* 2020, **140**:513–534.

66. Hou J, Zhou Y, Cai Z, Terekhova M, Swain A, Andhey PS, Guimaraes RM, Ulezko Antonova A, Qiu T, Sviben S, *et al.*: **Transcriptomic atlas and interaction networks of brain cells in mouse CNS demyelination and remyelination**. *Cell Rep* 2023, **42**, 112293.

67. ** Gouna G, Klose C, Bosch-Queralt M, Liu L, Gokce O, Schifferer M, Cantuti-Castelvetri L, Simons M: **TREM2-dependent lipid droplet biogenesis in phagocytes is required for remyelination**. *J Exp Med* 2021, **218**.

68. Depp C, Sun T, Sasmita AO, Spieth L, Berghoff SA, Nazarenko T, Overhoff K, Steixner-Kumar AA, Subramanian S, Arinrad S, *et al.*: **Myelin dysfunction drives amyloid-β deposition in models of Alzheimer's disease**. *Nature* 2023, **618**:349–357.
This study shows that when 5xFAD mice were crossed to a mouse with myelin deficits, microglia preferentially engulfed myelin over Aβ. Interestingly, the mice exhibited more amyloid beta deposition and fewer plaque-associated microglia than 5xFAD mice without additional myelin deficits.

69. Marschallinger J, Iram T, Zardeneta M, Lee SE, Lehallier B, Haney MS, Pluvinage JV, Mathur V, Hahn O, Morgens DW, *et al.*: **Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain**. *Nat Neurosci* 2020, **23**:194–208.

70. Cantuti-Castelvetri L, Fitzner D, Bosch-Queralt M, Weil M-T, Su M, Sen P, Ruhwedel T, Mitkovski M, Trendelenburg G, Lütjohann D, *et al.*: **Defective cholesterol clearance limits remyelination in the aged central nervous system**. *Science* 2018, **359**:684–688.

71. Safaiyan S, Kannaiyan N, Snaidero N, Brioschi S, Biber K, Yona S, Edinger AL, Jung S, Rossner MJ, Simons M: **Age-related myelin degradation burdens the clearance function of microglia during aging**. *Nat Neurosci* 2016, **19**:995–998.

72. Safaiyan S, Besson-Girard S, Kaya T, Cantuti-Castelvetri L, Liu L, Ji H, Schifferer M, Gouna G, Usifo F, Kannaiyan N, *et al.*: **White matter aging drives microglial diversity**. *Neuron* 2021, **109**:1100–1117 e1110.

73. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, *et al.*: **The classical complement cascade mediates CNS synapse elimination**. *Cell* 2007, **131**:1164–1178.

74. Filipello F, Morini R, Corradini I, Zerbi V, Canzi A, Michalski B, Erreni M, Markicevic M, Starvaggi-Cucuzza C, Otero K, *et al.*: **The microglial innate immune receptor TREM2 is required for synapse elimination and normal brain connectivity**. *Immunity* 2018, **48**:979–991.e978.

75. Hughes AN, Appel B: **Microglia phagocytose myelin sheaths to modify developmental myelination**. *Nat Neurosci* 2020, **23**:1055–1066.

76. Sarlus H, Heneka MT: **Microglia in Alzheimer's disease**. *J Clin Invest* 2017, **127**:3240–3249.

77. Merlini M, Rafalski VA, Rios Coronado PE, Gill TM, Ellisman M, Muthukumar G, Subramanian KS, Ryu JK, Syme CA, Davalos D,

et al.: **Fibrinogen induces microglia-mediated spine elimination and cognitive impairment in an Alzheimer's disease model**. *Neuron* 2019, **101**:1099–1108 e1096.

78. Lim TK, Ruthazer ES: **Microglial trogocytosis and the complement system regulate axonal pruning in vivo**. *Elife* 2021, **10**.

79. Weinhard L, Di Bartolomei G, Bolasco G, Machado P, Schieber NL, Neniskyte U, Exiga M, Vadisiute A, Raggioli A, Schertel A, *et al.*: **Microglia remodel synapses by presynaptic trogocytosis and spine head filopodia induction**. *Nat Commun* 2018, **9**.

80. Li Q, Haney MS: **The role of glia in protein aggregation**. *Neurobiol Dis* 2020, **143**, 105015.

81. Logan MA, Freeman MR: **The scoop on the fly brain: glial engulfment functions in Drosophila**. *Neuron Glia Biol* 2007, **3**: 63–74.

82. Lyons DA, Talbot WS: **Glial cell development and function in zebrafish**. *Cold Spring Harbor Perspect Biol* 2014, **7**, a020586.

83. Raiders S, Han T, Scott-Hewitt N, Kucenas S, Lew D, Logan MA, Singhvi A: **Engulfed by glia: glial pruning in development, function, and injury across species**. *J Neurosci* 2021, **41**:823–833.