Microglia-mediated synaptic pruning as a key deficit in neurodevelopmental disorders: Hype or hope?
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Abstract
There is a consensus in the field that microglia play a prominent role in neurodevelopmental processes like synaptic pruning and neuronal network maturation. Thus, a current momentum of associating microglia deficits with neurodevelopmental disorders (NDDs) emerged. This concept is challenged by rodent studies and clinical data. Intriguingly, reduced numbers of microglia or altered microglial functions do not necessarily lead to overt NDD phenotypes, and neuropsychiatric symptoms seem to develop primarily in adulthood. Hence, it remains open for discussion whether microglia are truly indispensable for healthy neurodevelopment. Here, we critically discuss the role of microglia in synaptic pruning and highlight area- and age dependency. We propose an updated model of microglia-mediated synaptic pruning in the context of NDDs and discuss the potential of targeting microglia for treatment of these disorders.

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Microglia, Neurodevelopmental disorders, Synaptic pruning, Immune system, CNS.

Introduction
For a long time, disorders of the brain have been approached with a central focus on neurons. However, it is becoming more and more evident that glial cells play important roles in brain function as well, for example in neuronal survival and synaptic plasticity [1]. Especially microglia, the resident immune cells of the central nervous system (CNS) have recently gained increasing attention for their role in neurodevelopment [2]. Traditionally viewed as the ‘trashcan’ of the brain, the field has now updated microglia to being among the most versatile cells of the body and involved in development and maintenance of brain circuitries and synaptic connections [3–5].

Microglia have highly motile processes allowing them to continuously scan their environment, interact with other cells, and exhibit inflammatory functions upon response to damage or infections [3]. Further, microglia control neuronal activity [6], modulate synaptic transmission [7], and shape connectivity in specific brain regions in response to sensory experience [8]. Next to these key roles in neuronal network function, there is particular interest in the role of microglia in synaptic pruning – an essential neurodevelopmental process in which excess synapses are eliminated. This mechanism allows for removal of redundant connections and thus circuit specialisation. In 2011, Paolicelli et al. showed for the first time that rodent microglia engulfed synaptic material in physiological conditions [9], implying that microglia-mediated synaptic pruning is part of healthy brain development. Nowadays, the role of microglia in synaptic pruning has been widely established [1,4], and multiple regulators of neuron–microglia communication have been discovered. These include well-known players of the peripheral immune system, such as cytokines, chemokines, and complement factors like C3 [10]. The complement receptor 3 (CR3), composed of CD11b and CD18, expressed on microglia recognises C3 localised at synapses which initiates synapse engulfment by microglia [11]. More recently, it has been found that externalised phosphatidylserine (PS) mediates synaptic pruning signalling on the neuronal side and interacts with the microglial receptor GPR56 and the complement component C1q [12–14] (Figure 1). A detailed overview of the regulatory mechanisms of synaptic pruning can be read in a recent review by Faust et al. [15].

Whether microglia engulf pre-synaptic-, post-synaptic material, or both, poses an ongoing discussion in the
In 2018, Weinhard et al. conducted light sheet live imaging to show direct and selective engulfment of the fluorescently labelled presynaptic protein synapsin in rodent microglia [16]. Contrary to previous assumptions, these data suggest that microglia selectively engulf presynaptic structures rather than the entire synapse [16]. What exactly distinguishes this experiment from other models showing postsynaptic material in microglia [9,11] needs to be determined. Potential explanations could be area and context specificity, which will be further discussed later. Additionally, the presence of postsynaptic material in microglia per se is not a definitive indicator of targeted synaptic pruning. An alternative route, where neurons release vesicles containing postsynaptic material and microglia phagocytose, these cannot be excluded without live-cell imaging techniques.

Currently, there is a momentum of associating microglia-mediated deficits in synaptic pruning with neurodevelopmental disorders (NDDs) [2,15]. In the traditional model, it is believed that over- or underpruning of synapses by microglia gives rise to NDDs (Figure 2). This potential involvement of microglia in NDDs has generated a wave of hope for novel treatment strategies, for example, microglial replacement therapies. In this review, we critically discuss the role of microglia in neurodevelopment and their involvement in NDDs.

**Synaptic pruning by microglia: can you do without?**

Given the consensus that microglia play a key role in synaptic pruning, the hypothesis that microglia deficits cause over- or underpruning of synapses manifesting in NDDs emerged [1,2,4,5]. This concept is challenged by rodent studies with reduced microglia numbers. Mice with deficient expression of TGFβ in the CNS, a cytokine required for microglia development, have a substantial loss of microglia. However, these mice develop normally until 100 days of age and show no overt NDD phenotypes [17]. Another cytokine important for the development of microglia is IL-34, which is produced by neurons in the CNS. Mice lacking IL-34 showed low abundance of microglia but did not display any substantially altered CNS phenotypes under steady conditions [18]. Considering this intriguing data, deficits in microglia-mediated synaptic pruning might not necessarily be causative of NDDs. Thus, the question arises whether microglia are truly indispensable for early neurodevelopment.
In addition to microglia, astrocytes are increasingly recognised for their role in synaptic function and are considered a central factor in neuronal network maintenance [19]. A landmark study in 2013 showed that astrocytes are also capable of engulfing synapses in the retinogeniculate system in mice in vivo [20]. This might imply that microglia and astrocytes prune in a collaborative manner. Damisah et al. investigated the phagocytic clearance of neuronal material and showed that both microglia and astrocytes are involved in this process [21]. However, microglia acted with extreme precision and specifically engulfed dendritic branches close to the soma whereas astrocytes engulfed large numbers of small fragments derived from more distal neuronal branches. This indicates that while both cell types are capable of phagocytosis, they have specialised roles with distinct territories [21]. In addition, microglia-mediated pruning mechanisms might be different from astrocyte-mediated mechanisms. For example, in contrast to microglial synaptic engulfment, astrocytic engulfment is independent of the complement protein C1q [20].

Based on these results, we propose that in the event of microglia absence or dysfunction, astrocytes might take over the phagocytic territories and additional functions of microglia to maintain synaptic homeostasis. Supporting this hypothesis, several studies found altered astrocyte activity in case functional microglia were absent. Depletion of microglia led to enhanced endocytosis by astrocytes [22] and disruption of intracellular communication between astrocytes [7]. Dysfunctional microglia resulted in elevated phagocytosis by astrocytes [23]. This indicates microglia-dependent changes in astrocytic function, potentially reflecting compensatory mechanisms to counteract pruning deficits due to microglia loss. However, astrocytic phagocytosis shows a slower degradation of material than microglia [21]. Hence, we propose a framework in which astrocytes take over microglial functions but are less efficient and not as specialised in refined synaptic pruning [15]. When the neuronal system is challenged, the absence of functional microglia might lead to abnormal neuronal network formation and impaired synaptic development manifesting in neurodevelopmental disorder phenotypes.

Area and age matters

A substantial amount of our understanding of the role of microglia in developmental synaptic pruning is based on work in the retinogeniculate system or olfactory bulb of rodents [8,12,13,24,25]. Data from these specialised brain regions with high levels of plasticity have contributed greatly to mechanistic insights into microglia-mediated synaptic pruning. However, dependent on the brain region, synapse elimination takes place with different time courses [26]. Further, there is an emerging concept that microglia-mediated synaptic pruning is region- and context-specific [15]. RNA sequencing data revealed that microglia display phenotypical heterogeneity across brain regions [27,28]. A sensory lesioning study showed that different brain areas utilise different signalling mechanisms of synapse elimination by microglia [29]. Considering this evidence, we need to acknowledge area specificity of microglial function in synaptic pruning and avoid oversimplifying findings from highly specialised brain regions. The circuit-dependent fashion of microglia-mediated synapse elimination could partially account for the absence of whole-brain deficits or severe neurodevelopmental phenotypes in case of microglia reduction or dysfunction.

In addition to area-specificity, there is age dependence. Morphologically, microglia change from more amoeboid to ramified cells during the course of brain development. As the developing brain faces challenges distinct from the adult brain, microglia might exhibit different functions during specific time points in neurodevelopment [4]. Indeed, single-cell analysis revealed distinct microglial gene expression patterns in foetal and adult human microglia, indicating functional differences in microglia between the developing and mature brain [30]. This has direct implications for NDDs. Broadly speaking, NDDs can be classified into two distinct groups depending on their typical onset. Intellectual disability (ID), autism spectrum disorder (ASD), and attention-deficit hyperactivity disorder (ADHD) have an early onset of symptoms, whereas in schizophrenia (SCZ), symptoms become evident later in adolescence.
In microglia-mediated disorders, such as hereditary diffuse leukoencephalopathy with spheroids (HDLS) [31] and Nasu-Hakola disease [32,33], symptoms typically manifest in adulthood. HDLS is a neurodegenerative disorder characterised by white matter abnormalities and glial neuropathology [31]. Individuals with HDLS carry autosomal dominant mutations in the colony-stimulating factor 1 receptor gene (CSF1R). Signalling of CSF1R is essential for microglial proliferation and function both during development and in response to assaults [34,35]. Accordingly, HDLS belongs to the class of primary microglial disorders, also known as microgliopathies [31]. Blocking of CSF1R with the inhibitor PLX3397 led to reduced pruning of excess synapses in the rodent auditory brainstem [36], indicating a requirement of CSF1R activation for microglia-mediated synaptic pruning. These results suggest that individuals with HDLS have an altered microglial function and potential deficits in microglia-mediated synaptic pruning. Interestingly, the average
onset of HDLS is 43.3 years, ranging from 10 to 71 years, and these patients do not show NDD phenotypes like ASD or ID [37]. Nasu-Hakola disease, another microglialopathy, is associated with a loss of function of the triggering receptor expressed on myeloid cells 2 (TREM2) or its adaptor protein TYRO protein tyrosine kinase binding protein (TYROBP) [32,33]. In the absence of TREM2, microglia show reduced engulfment capacities, and there is an increased number of synaptic contacts indicating inhibited synapse elimination [12,38]. Notably, Nasu-Hakola disease is characterised by dementia and demyelination with an onset in adolescence to adulthood [33].

This association between genetic variation in microglia-related genes and neurological and psychiatric clinical phenotypes may provide indications about the role of microglia in NDDs. Variants in TREM2, but also many other well-known microglia genes, have been primarily linked to neurodegenerative disorders, like Alzheimer’s and Parkinson’s disease instead of early onset NDDs [28,38–40]. Copy number variants in the complement C4A gene have been associated with schizophrenia [41,42] and overexpressing the complement component 4 (C4) in rodents led to increased synapse engulfment by microglia resulting in abnormal brain circuits and behaviour [43]. The microglial receptor for fractalkine, CX3CR1, has been established as a key mediator in microglia-mediated synaptic pruning [9,29], and polymorphisms in CX3CR1 are also associated with SCZ [44]. When evaluating the highly confident ASD and ID genes, there are no hits in genes typically associated with microglial function [45,46] (https://gene.sfari.org/). Taken together, genes involved in microglial function are primarily associated with adolescence-onset NDDs or neurodegenerative disorders. Thus, we hypothesise that microglia-mediated synaptic pruning plays a more prominent role in NDDs where symptoms manifest later in life, like SCZ.

**Microglia: saviours in challenging times?**

Mutations in classical microglial genes have no clear association with early onset NDDs. In fact, most of the ID/ASD-related genes have been related to synaptic plasticity and function, or to more generalised functions, such as epigenetic regulation [47–50]. Does this mean that microglia do not contribute to NDDs that present early in life? Patients with early onset NDDs often present with a second phase of new symptoms in adolescence, such as mood symptoms, psychosis, and cognitive decline. In addition, disease severity and symptomatology are largely variable. We speculate that microglia may contribute to this variation and development of these adolescent-onset symptoms in NDDs. Due to their responsive nature, microglia can rapidly react to stress, inflammation, or high levels of neuronal activity and thereby contribute to maintain homoeostasis in the neuronal system. For instance, several recent studies have shown a protective role of microglia in epilepsy. Microglia depletion exacerbated seizure severity [51] as well as seizure frequency and duration [52]. Further, it was shown that microglia position their processes around neuronal dendrites in case of severe seizures, potentially acting in a neuroprotective manner [53]. These studies highlight the contribution of microglia in challenged neuronal systems, like epileptic overexcitation. In addition, microglia may respond to the neuronal deficits that are underlying the NDD, and this response may either have a beneficial or detrimental outcome. In line with this, microglial changes have been reported in post-mortem brain tissue of individuals with ASD [54,55]. Also, in monogenetic forms of NDDs, changes in microglia phenotype and function have been found. Altering the cellular localisation of Pten, a gene associated with ASD led to enhanced synapse engulfment by microglia [56]. In addition, models for Rett syndrome [57], Tuberous Sclerosis Complex [58], Fragile X syndrome [59], and Down syndrome [60] have shown alterations in microglial cells, including altered numbers, transcriptome profiles, morphologies, responses to cytokines, phagocytosis of extracellular material.

Interestingly, synaptic pruning regulation intersects with typical signalling molecules of myeloid cells to detect and respond to danger, like the complement system [11,10,61]. This might constitute a clever ‘double function’ of microglia signalling, enabling microglia-mediated synaptic pruning to happen precisely at the right times and locations during emergency situations. Hence, we speculate that microglia-mediated synaptic pruning becomes particularly important when the neuronal system faces challenges like stress, infections, or neurotoxicity. Thus, in patients with an early onset NDD microglia might play a key role in variation between patients, as well as for NDD symptoms manifesting later in life. However, whether microglia play a beneficial or detrimental role in these NDDs is still debated, and probably depends on the timing, region, additional challenges, and the underlying deficit.

**Conclusion and future outlook**

The majority of studies investigating microglia-mediated synaptic pruning were conducted in rodents [38,29,62,8,13,12,63,25,59,56,14,64]. This animal work contributed significantly to our current knowledge; however, species-specific variations in microglia should be considered when interpreting results. There are substantial differences in human and rodent microglia, like turnover and maintenance, diversity along spatial and developmental dimensions, and responses to stimulatory cues [65]. Further, transcriptomic analysis revealed an overlap of human and murine homoeostatic
microglial gene expression of only >50% [65]. To bridge this gap, relevant human models should be included in the studies of microglia-mediated synaptic pruning. The latest advances in the field of induced pluripotent stem cell (iPSC) technology allow for in vitro modelling of patient-specific cells, including microglia [65]. One recent example applying this technology showed that microglia derived from schizophrenia patients phagocytosed significantly more synaptosomes compared to microglia from healthy controls [66]. Further, 3D modelling of microglia-neuron interactions in iPSC-derived organoids will help to unravel the role of microglia in neuronal network function [67].

The growing recognition of microglia in engulfment of synaptic material created a momentum of associating microglia-mediated synaptic pruning deficits to NDDs. In this paper, we refined this idea by considering data from microglia-deficient mice and patients with microgliopathies. Despite altered microglial function, HDLS patients do not show NDD phenotypes and develop neurological symptoms in late adulthood. We discussed potential compensation by other glial cells, the tipping point theory, and area specificity as explanatory mechanisms for this intriguing data. Furthermore, altered microglia-neuron crosstalk might be underlying NDDs rather than a dysfunction of microglia per se. The consequences of dysfunctional microglia during neurodevelopment might be distinct from a scenario in which microglia over-, under-, or dys-prune synapses as a consequence of changes in the neuron-microglia crosstalk. Pruning deficits can originate from both the neuronal signalling and the microglial side, which has implications for novel therapeutic strategies like microglial replacement therapies. These provide attractive opportunities for treatment of leukoencephalopathy [68–70]. In a recent study, microglial replacement therapy via stem cell transplantation ameliorated symptoms in a mouse model of progressive neurodegeneration [71]. While microglial replacement therapy is of great potential for neurodegenerative disorders and adult-onset microgliopathies, the picture in NDDs might be more complex. Lacking microglia does not necessarily result in NDDs and if the crosstalk between neurons and microglia is altered, simply replacing microglia might not be the most promising solution.

We propose an updated model which (i) centralises the nature of pruned synapses instead of the amount of synapses engulfed and (ii) highlights the context- and age dependence of microglial function (Figure 4). Eliminating precisely those connections that are nonfunctional or redundant is crucial for neuronal network development. We speculate that the absolute quantity of pruned synapses could be identical between disease context and physiological situation, but which synapses exactly are

Figure 4

Updated model of synaptic pruning and the role of microglia during neurodevelopment. We propose an updated model which centralises the nature of pruned synapses instead of the amount of synapses engulfed and highlights area-, age-, and context dependence of microglial function. The absolute quantity of pruned synapses could be identical between disease context and physiological situation, but which synapses exactly are pruned defines the phenotypical outcome. There is an additional role of astrocytes in the basic pruning process. However, during challenging conditions synaptic pruning becomes increasingly microglia-dependent to maintain homeostasis. Microglia are particularly important for synaptic pruning in late adolescence/adulthood and might determine the presence of adult-onset symptoms in NDDs.
pruned defines the phenotypical outcome. Given the key role of microglia during stress, we speculate that synaptic pruning becomes increasingly microglia-dependent in challenging environments, whereas cell-intrinsic mechanisms and astrocytes contribute to pruning during basal conditions. From evaluating microglial genes associated with NDDs and considering onsets of disorders related to microglia deficits, we conclude that microglia contribute primarily to adolescent-onset psychiatric symptoms and neurodegenerative disorders. Although we challenge the concept of microglia as key players in the entire spectrum of NDDs, our updated view also provides hope. The fact that microglia may especially play a role in dysregulated synaptic pruning during adolescence provides a window of opportunity to diminish this process and prevent symptoms from manifesting later in life. In the future, we should move beyond discussing whether too many or too few microglia are detrimental for neurodevelopment and shift the focus towards how functional characteristics of microglia and their crosstalk with neurons in different contexts, areas, and ages contribute to NDDs.

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Conflict of interest
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Data availability
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References
Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest


This rodent study shows that in case of dysfunction of microglia, astrocytes upregulate their phagocytosis and show pro-inflammatory gene expression profiles. These compensatory mechanisms might be crucial for CNS homeostasis and support our concept of an astrocytic role in synaptic pruning when microglia are dysfunctional or absent.


This review summarises phenotypes linked to mutations in the colony-stimulating factor 1 receptor gene (CSF1R). Signaling of CSF1R is essential for microglial proliferation and function both during development and in response to assaults. Thus, CSF1R-related disorders are classified as primary microglial disorders. Interestingly, even though these patients display microglia with altered function, the average onset of symptoms is in adulthood and no overt neurodevelopmental disease phenotypes are observed.


Microglia-mediated synaptic pruning
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