

Microglia and early brain development: an intimate journey

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ABSTRACT

Crosstalk between the nervous and immune systems has been well described in the context of adult physiology and disease. Recent advances in understanding of immune cell ontogeny have revealed a remarkable interplay between neurons and microglia during the prenatal and postnatal emergence of functional circuits. This review focuses on the brain, where the early symbiotic relationship between microglia and neuronal cells critically regulates wiring, contributes to sex-specific differences in neural circuits, and relays crucial information from the periphery, including signals derived from the microbiota. These observations underscore the importance of studying neurodevelopment as part of a broader framework that considers nervous system interactions with microglia in a whole-body context.

INTRODUCTION

The standard classification of central nervous system (CNS) lineages separates neurons that process information, from glial cells, which support and modulate neuronal activity. Glial cells are now beginning to emerge as major contributors to CNS development and homeostasis. In particular, brain-resident macrophages termed 'microglia' appear to play major roles at the interface between the immune and nervous systems **(1-5)**, each of which is crucial for host perception of the external environment. Both the nervous and immune systems share a number of key attributes, including the need for a critical training period during which baseline levels of input are defined, such that subsequent atypical and potentially dangerous signals can be detected efficiently. Despite these common features, the nervous and immune systems have classically been studied independently. However, there is now increasing evidence of a strong neuro-immune interplay in adults that determines how the brain modulates tissue immunity and normal processes of ageing, as well as inflammation and neurological disorders including Alzheimer's disease, Parkinson's disease, Multiple Sclerosis and various autoimmune pathologies **(1-4)**. In addition, activation of the immune system during pregnancy or early-life has been shown to exert long-term effects on the wiring of neural circuits and may contribute to the etiology of neurodevelopmental disorders. Microglia develop in close proximity to neurons and other glial cells from early embryonic stages, and multiple studies have now established a pivotal role for these cells in CNS development and homeostasis. This review attempts to highlight important recent advances in this field, focusing on the developmental roles of microglia at the interface between brain circuits and the periphery, while also identifying key goals for future research.

Ontogeny and developmental trajectories of CNS immune cells

Microglia represent 5 to 15% of adult brain cells, with densities varying between distinct brain regions. They constitute by far the largest population of immune cells in the brain, and under physiological conditions they are unique in being located within the brain parenchyma, where they lie in direct contact with neural progenitors, neurons, and other glial cells (namely astrocytes and oligodendrocytes). The genesis of microglia remained obscure for many years, until landmark studies in mammals identified a very early embryonic origin for this population within the yolk-sac (YS) **(1, 2, 5)**. This earliest primitive wave of hematopoiesis arises at embryonic day (E) 7.5 in mice, generating nucleated red blood cells and macrophages that colonize the entire embryo. Macrophages start to enter the CNS at E9 using the blood vasculature prior to closure of the blood-brain-barrier (BBB) which occurs around E13 in mice, restricting access to immune cells that arise later in development **(5) (Fig. 1)**. At these early stages, neural progenitors are actively dividing and generate the first neurons, whereas they will give rise to oligodendrocytes and astrocytes only at late embryonic timepoints. Consequently, microglia constitute the main glial population during a large part of fetal life.

In most tissues located outside of the CNS, yolk sac-derived macrophages are progressively replenished from circulating monocytes, which are generated later in development from fetal and definitive bone-marrow hematopoiesis **(1, 2, 5)**. In contrast, microglia, which are located behind the BBB, self-renew for the entire life of the animal, at least under steady-state conditions. It is important to note that this situation is different in zebrafish, in which secondary waves of hematopoiesis contribute to the microglial pool. Yolk sac-derived macrophages also colonize the meninges and other surrounding CNS tissues to give rise to border associated macrophages (BAMs),

including meningeal, choroid plexus and perivascular macrophages (PVM) **(6)** **(Fig. 1)**. However, future studies will be needed to determine whether these arise from common precursors, to characterize their potential differentiation pathways and divergent functions. In other words, it remains unclear whether microglia vs BAM lineage commitment depends on specific precursor populations generated in the YS, or whether these distinct fates are instructed by local cues encountered by macrophages as they travel through the developing CNS. In contrast, choroid plexus macrophages are known to be replaced over time by cells generated during adulthood, supporting the notion that the choroid plexus lacks a discernable BBB and thus remains accessible to circulating bone marrow-derived cells during later life **(6)**.

In adult mice, other immune populations including monocytes and lymphocytes, which lie outside of the parenchyma, have been shown to regulate neural function via the production of long-range signals including IL-4, TNF α and IFN γ **(7-9)**. However, there are only few studies to suggest that equivalent populations influence the developing brain. In parallel, recent evidence has shown that the neonatal mouse parenchyma can be infiltrated by B cells even in physiological conditions, with an influence onto oligodendrogenesis **(10)**. Future studies will therefore be essential to decipher the potential impact of different immune cell populations on brain development, whether acting indirectly via effects on microglia or capable of directly influencing neurons.

In any event, the developing CNS clearly has a unique immune status associated with the presence of long-lived resident macrophages that originate in early embryogenesis **(Fig. 1)**. These data indicate that the microglial population has a life-long history, potential memory of previous interactions, and a unique symbiotic relationship with the developing brain.

Neural cues and systemic signals shape microglial development

Colonization of the brain parenchyma by yolk sac-derived macrophages is a long-lasting process that spans embryogenesis and early postnatal stages (**1, 11, 12**). In the mouse, the first macrophages enter around E9 and the adult pattern of homogeneous tiling is reached during the second postnatal week (**1**), in paralleling the acquisition of a progressively more ramified microglial morphology. Transcriptomic and functional studies have revealed that, like other tissue-resident macrophages, microglia undergo distinct phases of differentiation (**13-16**) that rely on signals derived from the maturing CNS, including but not restricted to CSF1, IL-34 and TGF β (**1**) (**Fig. 2**). At the molecular level, transition between a brain macrophage signature and a microglial profile can be identified by the expression of core genes including the key transcription factor *Sall1* and purinergic receptor P2Y₁₂ (**1, 15, 16**). Deciphering the full range of signals that regulate this maturation process is extremely challenging given that microglia rapidly lose transcriptional and epigenetic identity upon separation from their niche (**15, 16**). In addition, the adult CNS environment is not sufficient to induce full microglial differentiation of bone marrow-derived monocytic cells (**17, 18**), indicating that both their early origin and interactions with the CNS are critical for the acquisition of a *bona fide* microglial identity.

In addition, local neural signals likely regulate the brain colonization pattern and heterogeneity of microglial populations in both time and space. Microglia enter the CNS via sequential waves (**11, 12**) and show transiently a stereotypical and heterogeneous pattern of localization (**11, 19, 20**). For instance, microglia undergo timely invasion into the deep layers of the neocortex, but they also associate with distinct axonal tracts including the corpus callosum and dopaminergic midbrain axons, as well as populating

specific niches known to contain neurogenic progenitors **(20)**. This distinctive pattern is thought to be regulated by transient CNS cell expression of cues including IL-34, CSF1, CXCL12 and CX3CL1/fractalkine **(1) (Fig.2)**. In addition, studies in zebrafish have indicated that developmental programmed cell death in the CNS regulates microglial colonization **(1)**. Following early colonization, there is increasing evidence that adult microglia display some heterogeneity across brain regions, particularly in the corpus callosum, prefrontal cortex, cerebellum or basal ganglia **(1, 12, 21, 22)**. Indeed, transcriptomic profiling, genetic labeling and analysis of cell dynamics have revealed a degree of heterogeneity, suggest that regional differences may arise in part due to local signals produced by neural cells **(1, 12, 21, 22)**. Future single cell transcriptomic studies will be required to unravel this heterogeneity and determine the underlying mechanisms. Nevertheless, the studies conducted to date indicate that CNS-derived signals have a major role to play in the specification, maturation and colonization of microglial populations **(Fig. 2)**.

In addition to local cues, the developmental trajectory of microglia is known to be influenced by sexual identity and systemic signals such as those derived from the microbiota or inflammation at pre- and postnatal stages **(14, 23)**. Several reports have highlighted a distinct transcriptomic signature of microglia in males versus female animals that begins postnatally and is maintained in adults **(14, 23, 24)**. This sexual dimorphism appears to be conserved after grafting and may occur independently of circulating sex hormones, suggesting long-lasting programming by early-life hormones or genetic factors **(24)**. Barrier organs including the gut, skin and lungs are also populated by a diverse range of bacteria and other microorganisms termed the 'microbiota', which plays essential roles in the development and training of both innate and adaptive immune systems **(25)**. Despite their relative isolation behind the BBB,

microglia are able to respond to microbiota-derived signals both pre- and postnatally **(13, 14, 26)**, since germ-free mice display incomplete development of microglia both during embryogenesis and at birth **(13, 14)**. While in adults the microbiota has been shown to act on microglia in part via systemic production of short chain fatty acids (SCFA) **(26)**, the mechanisms involved remain unexplored in embryos. Conversely, inflammation is also known to change microglial developmental trajectory, from early embryonic stages **(13, 23)** into adulthood, in particular via the production of cytokines that can act over long distances. Intriguingly, microglial responses to inflammation and microbiota-derived signals differ between females and males **(14)**, although the underlying mechanisms remain to be defined. As the list of signals that can influence microglial development continues to expand, it becomes increasingly clear that these cells are capable of integrating a diverse range of systemic and local cues that likely have significant implications for neuronal function.

At a mechanistic level, it will be essential for future studies to determine how microglia can integrate such a wide variety of influences. Recent analyses of conditional HDAC1 and 2 mutants have shown that embryonic epigenetic modifications are essential for full microglial differentiation **(27)**. Because the microglial population self-renews throughout life, somatic mutations occurring in the lineage or epigenetic modifications within this population, induced either by local or systemic signals, could have major physiological or even pathological consequences in adults **(15, 16, 28)**. For instance, somatic mutations in primitive hematopoiesis in mice were shown to induce neurodegeneration in adults **(28)**. It will therefore be important to assess if and how genetic and epigenetic changes during the critical developmental period regulate the emergence of correctly-tuned microglia and brain-resident immune cells.

The multifaceted roles of microglia in early CNS development

While the overall morphogenesis of brain structures and their assembly do not require microglia, a number of recent studies have reported a central role for these cells in specific aspects of brain development, homeostasis and disease. In particular, microglia were shown to eliminate apoptotic cell debris and contribute to activity-dependent synaptic reorganization, with a role in presynaptic nibbling and promotion of post-synaptic spine formation **(2-4, 29, 30)**. This later process has already been the focus of landmark reviews **(1-4)** and is regulated by the complement cascade, TREM2 **(31)** or by the CX3CL1-CX3CR1 receptor pathway, depending on the stage and structure concerned. Importantly, these studies link microglia dysfunction with behavioral deficits in adults as well as neuropsychiatric disorders.

Consistent with their unique early ontogeny and uneven pattern of brain colonization, several additional functions of microglia during neuronal development are now beginning to emerge **(Fig. 3)**. Microglia have been linked with the regulation of neuronal numbers, the early wiring of neural circuits, and the acquisition of sex-specific features. Indeed, microglia show a remarkable localization to several neurogenic niches in the rat brain, including the postnatal subventricular zone and neocortex, where they regulate non-apical progenitor cell numbers via selective engulfment **(Fig. 3A)(20)**. This situation has also been observed in the mouse but appears less pronounced, suggesting that these events may display species specificity **(1)**. In addition, microglia are also emerging as regulators of early circuit assembly via the CX3CL1-CX3CR1 pathway. In normal physiology, microglia transiently associate with specific axonal tracts, including dopaminergic axons and corpus callosum axons, where they then regulate axon progression and fasciculation **(Fig. 3B)(19, 32)**. Microglia can also regulate the migration of inhibitory cortical interneurons that are

essential regulators of excitation/inhibition balance and which dysfunctions have been implicated in autism spectrum disorders (ASD) and schizophrenia **(Fig. 3C)(19)**. Independently to this early role in wiring, a subset of white matter microglia have been reported to promote survival of layer 5 pyramidal neurons via the production of IGF1 **(Fig. 3D)(33)**, supporting the idea that microglia can influence neuronal numbers at distinct steps and through multiple mechanisms. In addition, early postnatal microglia have also been reported to contribute to masculinization of the preoptic area, releasing prostaglandins and regulating connectivity, thereby inducing male-specific copulatory behavior in the offspring **(Fig. 3E)(34)**. These findings reveal a remarkable interplay between steroid hormones, microglia, and the early acquisition of sexually-dimorphic brain circuitry and behavior. Whether these features also apply to other brain regions or time-periods such as adolescence remains entirely unknown.

In parallel with their roles in early neuronal development, microglia crosstalk with other glial cells that modulate neuronal function. Microglia have been reported to lie in close apposition with blood vessels and to modulate endothelial tip cell fusion **(1)**. Since microglia and PVM are difficult to discriminate at these early stages, further experiments will be required to clearly assess the relative contributions of each population. In addition, microglia are key regulators of oligodendrocyte precursor differentiation and myelin formation. In particular, subsets of microglia expressing CD11c promote myelination via IGF1 release, revealing a remarkable coordination of both neuronal survival and myelination by a single soluble factor **(Fig. 3D)(21, 22)**. Lastly, astrocyte-derived IL-33 was recently shown to regulate microglial phagocytosis of neuronal spines **(Fig. 3E)(35)**, clearly demonstrating a neuronal output to astrocyte-microglial interactions.

Together, these studies establish microglia as central mediators of crosstalk between neuronal and glial cells, where they regulate multiple aspects of early brain wiring in addition to their well-characterized roles in synaptic remodeling. Importantly, while specific molecular pathways control microglial activity and live imaging reveals dynamic physical interactions with their CNS neighbors, we still have a limited understanding of how microglia act and exert specific functions. Combining *in vivo* imaging, single cell spatial information, and identification of the key molecules involved via transcriptomic studies will bridge the gap between the molecular and cellular levels and provide a much-needed conceptual framework for microglial functions.

Impact of inflammation on microglial and CNS development

In addition to local interactions between microglia and their CNS neighbors, long-range signaling from other embryonic tissues and even the maternal environment can modulate directly or indirectly developing CNS cells. For example, it is now well-recognized that systemic inflammation during pregnancy is associated with defective brain wiring. Epidemiological studies have revealed potential links between bacterial or viral infection during the first and second trimesters with an increased risk of ASD or schizophrenia in the offspring (**36**). Importantly, animal models of this Maternal Immune Activation (MIA) can be induced by injections of polyI:C (a synthetic analog of double strand RNA present in some viruses) or lipopolysaccharide (LPS, a component of the membrane of gram negative bacteria) in pregnant dams to respectively mimic viral or bacterial infections. Remarkably, these procedures are sufficient to induce a set of core behavioral deficits related to neurodevelopmental disorders in the adult offspring (**36, 37**). It is important to note that there are multiple MIA models described in the literature that employ different stimuli and injection protocols (dose / timing / frequency) which induce distinct behavioral alterations. In the well-studied MIA-polyI:C

model, the Patterson lab performed seminal work demonstrating an essential role for long-range signaling by IL-6 cytokine in MIA pathology (37). More recently, it was elegantly shown that IL-6-induced differentiation of maternal Th17 cells and subsequent production of IL-17 in the mother can signal directly to the placenta and embryo (38, 39). These two cytokines in particular appear to play key roles in mediating the deleterious effects of prenatal inflammation on embryonic wiring of the mouse brain. In humans, levels of IL-6 production in pregnant mothers have been correlated with functional connectivity and working memory in babies (40), suggesting a potential conservation of this mechanism between species.

How might circulating cytokines impact on fetal brain wiring? While it has been proposed that IL-17 could act directly on neurons (39), it is also well-established that microglia are perturbed by MIA and could thereby contribute to neuronal dysfunction (3, 13, 23, 36). A consistent finding is that MIA models and embryonic-stage depletion of microglia induce phenotypic similarities in the affected animals, in particular the distinctive dysregulation of cortical inhibitory interneurons (19), which is a hallmark of several neurodevelopmental disorders. In addition, MIA is known to induce long-term changes in the developmental trajectories of microglia (23). Since microglia are a long-lived population, early-life inflammatory events not only induce acute effects, but potentially also long-term modifications in brain wiring, plasticity and neurodegeneration (27). Mechanistically, it will be essential to assess the roles of epigenetic modifications, which regulate microglial differentiation and are susceptible to environmental signals (13, 14).

It is important to note here that maternal Th17 cells are known to be regulated by the composition of the microbiota, hence MIA-induced morphological and behavioral phenotypes are highly variable between offspring generated under different

housing conditions. Indeed, host Th17 cells appear strictly dependent on the presence of species including segmented filamentous bacteria (SFB) within the gut microbiota, but SFB are not present in all animal facilities used to generate MIA mouse models. These results highlight the urgent need to clarify the effects of various different MIA protocols, including the nature and timing of the microbial stimulus employed, the mouse strain used, and microbiota composition in different animals. They furthermore reveal the tight circular relationship between the microbiota composition, the systemic immune response and brain miswiring.

FUTURE DIRECTIONS

The study of neuron/microglia interactions has led to remarkable progress in our understanding of synaptic homeostasis and has major implications for knowledge of the ageing process and neurodegenerative diseases (**1-3, 5**). Additional key functions of microglia are emerging during early neurodevelopment albeit the underlining mechanisms and their direct contribution to neurodevelopmental disorders remain to be fully explored. Building novel tools and models to dissect these functions and assess their roles in neurodevelopmental disorders remains a major goal for the field. In particular, combining *in vivo* studies in animal models, brain organoids, iPSC-derived microglia (**1**), and CRISPR-CAS9 gene editing technology, will be instrumental for our comprehension of microglia/neuron/glia crosstalk in physiological and pathological conditions. Furthermore, deciphering the roles of microglia vs other immune populations lying inside and outside the parenchyma during CNS development will be important to grasp the logic of neuroimmune interactions and the deleterious effects of inflammation. Finally, while most prior studies have focused on the prenatal or early postnatal stages of life, little is known about neuro-immune interactions during adolescence, which constitutes a major period of brain circuit

rearrangements and a critical window of susceptibility to disorders including schizophrenia. In light of the sexually-dimorphic properties of immune cells and their potential to modulate brain wiring, it will be of particular interest to study this key developmental event. Early neuro-immune interactions not only forge circuits in the context of the whole body, sexual identity, and dialogue with the environment, they also program immune cells that persist in the brain throughout life. Defining the cellular and molecular basis of these interactions will be essential to advance our current understanding of brain homeostasis and pathology.

FIGURES

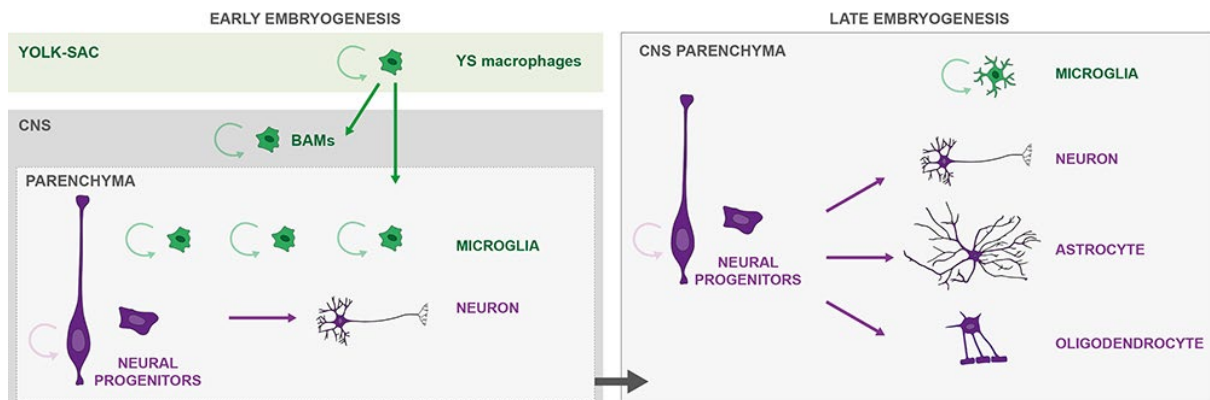


Figure 1. Parallel development of neural and immune cells of the CNS. Schematic showing early CNS colonization by yolk-sac derived macrophages that give rise to microglia and border associated macrophages (BAMs), whereas neural progenitors generate neurons (left). At later embryonic stages (right), neural progenitors additionally produce oligodendrocytes and astrocyte precursors in the parenchyma while the microglia undergo local differentiation. BAMs: border associated macrophages; CNS: central nervous system; YS: yolk-sac.

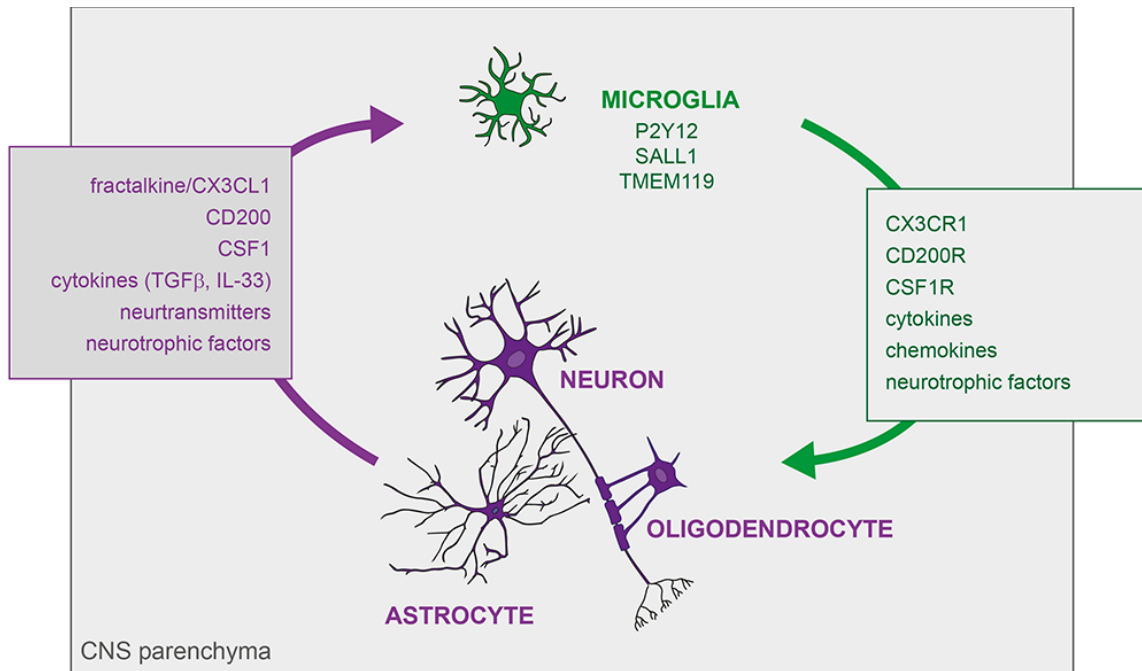


Figure 2. Crosstalk in the development of microglia and CNS cells. Respective expression of secreted or membrane-bound factors (boxes) are essential for neuro-immune dialogue and are instrumental in the acquisition of a microglia-specific signature as characterized by expression of CSF1R, Sall1, P2RY12 and Tmem119.

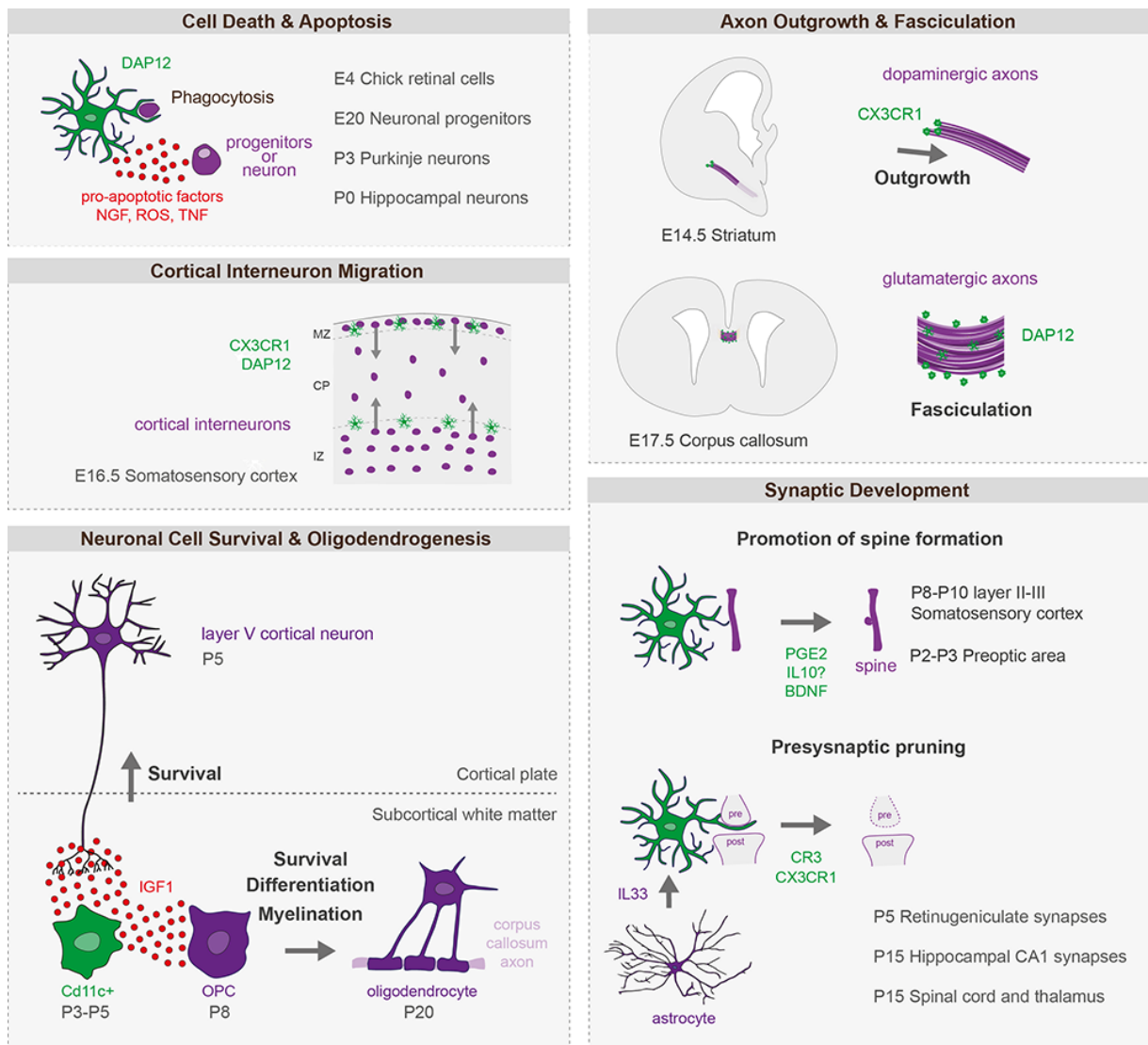


Figure 3. Main cellular functions of embryonic and post-natal microglia. Schematic representation of microglial functions during pre- and post-natal development. **(A)** Microglia act as regulators of neuronal cell number by actively phagocytosing progenitor cells and promoting apoptosis of differentiated cells via mechanisms including NGF secretion. **(B)** During pre- and postnatal development, microglia also participate in the emergence of connectivity by promoting outgrowth or fasciculation of axonal tracts such as dopaminergic and corpus callosum axons. **(C)** Microglia can further influence neuronal migratory processes and regulate the development of inhibitory interneurons in the embryonic somatosensory cortex. **(D)** During the first postnatal week, microglial release of IGF-1 is required to regulate layer

V pyramidal cell survival in the somatosensory cortex. Similarly, in the cerebellar white matter and corpus callosum of rodents, a neonatal subpopulation of CD11c^{high} microglia expresses large amounts of IGF-1 and thereby regulates oligodendrocyte precursors and myelination. **(E)** After birth, microglia are additionally involved in the promotion of excitatory synapses in the neocortex, either by promoting the formation of spines or regulating synaptic transmission. Microglia are well described mediators of synapse elimination through engulfment of presynaptic inputs, which rely on activity-dependent and complement tagging in the visual cortex and retinogeniculate system, as well as on CX3CR1 expression in the hippocampus. This role of microglia in synaptic pruning is closely regulated by neuronal activity, but also by astrocyte secretion of IL-33, as recently shown in the spinal cord and thalamus. Microglia are depicted in green, released factors in red, and targets in purple. For each situation, the developmental stage (embryonic, E or postnatal, P) and anatomical location are indicated. OPC, oligodendrocyte progenitor cell; PGE₂, prostaglandin E₂; MZ, marginal zone; CP, cortical plate; IZ, intermediate zone.

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