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Microglia: Architects of the Developing Nervous System

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Abstract

Microglia are resident macrophages of the central nervous system (CNS), representing 5–10% of total CNS cells. Recent findings reveal that microglia enter the embryonic brain, take up residence before the differentiation of other CNS cell types, and become critical regulators of CNS development. Here, we discuss exciting new work implicating microglia in a range of developmental processes including regulation of cell number and spatial patterning of CNS cells, myelination, and formation and refinement of neural circuits. Furthermore, we review studies suggesting that these cellular functions may result in modulation of behavior, which has important implications for a variety of neurological disorders.

Keywords

Microglia; Development; Central Nervous System

Microglia in the developing central nervous system

Development represents a remarkably dynamic window in the course of an organism's life, requiring coordination and communication among vastly different organ systems and cell types. In the central nervous system (CNS), a large variety of neurons and glial cells must communicate with one another to achieve the exquisite structure and function that are characteristic of the mature system. Included among these cell types are microglia, the resident brain macrophage, which make up approximately 5–10% of total CNS cells. Microglia are one of the first tissue macrophages to be born in the yolk sac at ~embryonic day 7.5 (E7.5) and migrate into the brain rudiment at ~E9.5 where they take up residence and self-renew throughout life [1–5]. The timing of this colonization occurs prior to the differentiation of other resident nervous system cells [6]. As a result, microglia are present at the right time and place to play critical roles in CNS development. Here, we review recent work demonstrating that microglia regulate an array of developmental processes that are necessary for achieving appropriate cellular architecture and function in the mature CNS.

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We also discuss emerging idea that these cellular functions may be novel mechanisms by which devastating neurological disorders manifest (Table 1).

Control of Neuron Cell Fate and Number

In the developing CNS, resident cells are born and migrate to their appropriate location. During this process, a subset of newly born cells must be lost during normal programmed cell death (NPCD) while the remaining cells mature [7, 8]. Early imaging studies demonstrated that microglia engulf dead or dying cells throughout the developing brain [9, 10] (Figure 1A). However, it remained unclear whether microglia played a more active role by initiating the cell death program prior to engulfment. Some of the most direct evidence for a more active role were in vitro studies in chick retina where NPCD was reduced when retinas were cultured in the absence of microglia [11]. When microglia were added back to the cultures, retinal cell death increased—an effect attributed to microglia-derived nerve growth factor (NGF). Similarly, in cultured mouse cerebellar slices or rat spinal cord explants, microglia engulfed dead or dying cells, and pharmacological depletion of microglia resulted in reduced Purkinje neuron and motoneuron NPCD [12, 13]. Superoxide ions released from microglia mediated Purkinje neuron cell death in the cerebellum, while microglial-derived TNF-α initiated NPCD of motoneurons in the spinal cord. These data suggest that microglia not only play a critical role in clearing cellular debris of dead or dying cells, but also actively initiate the cell death program.

Similar to in vitro studies, microglia have been suggested to regulate NPCD at sites of neurogenesis in vivo. In developing zebrafish, phosphatidyl serine receptors, Bal1 and Tim-4, were recently identified to regulate the phagocytic machinery necessary for microglia to clear dying neurons in the developing brain [14]. However, apoptosis still progressed in the absence of microglia. In macaque monkeys and rats, microglia engulfed excess neural progenitor cells (NPCs) as neurogenesis neared completion in the cerebral cortex [15]. Furthermore, the number of cortical NPCs increased when microglia were pharmacologically inactivated with broad spectrum antibiotics (minocycline or doxycycline) or depleted with liposomal clodronate [15]. Conversely, treating mice in utero with lipopolysaccharide (LPS) to increase the inflammatory state of microglia resulted in a decrease in NPCs in the cortex. These data suggest that microglia regulate NPC number by initiating cell death in the mammalian brain and engulfing dead or dying cells. Given that the pharmacological approaches in the mammal are relatively non-specific, future work is necessary to determine if these effects are microglia specific. In addition, it is unknown whether Bal1 and Tim-4-dependent phagocytosis of dying neurons in the developing zebrafish is a conserved mechanism across species.

Microglial-derived factors are also critical to survival, proliferation, and maturation of NPCs in the developing brain. For example, the addition of microglia-conditioned media to cultured neurons resulted in an increase in NPC proliferation coupled with enhanced neuron survival and maturation [16–18]. Similarly, NPCs isolated from embryonic day 12 (E12) mice that lack microglia (PU.1-deficient mice) exhibited decreases in both proliferation and astrogenesis, effects that were attenuated by the addition of wild-type microglia [19]. In contrast, NPCs isolated from 3 month-old rats and co-cultured with increasing

concentrations of microglia, revealed an inverse correlation between progenitor cell survival and microglia concentration [20]. These different conclusions may be a result of regional differences in microglia function (cortex vs. hippocampus) or differences in culture preparation. To assess whether microglia provide trophic support to neurons in vivo, genetic mouse models have been utilized. Mice deficient in the fractalkine receptor (CX3CR1), a chemokine receptor highly enriched in microglia in healthy CNS, had significant increases in numbers of apoptotic neurons in layer V of the postnatal cerebral cortex [21]. This effect was replicated by pharmacologically inactivating or genetically depleting microglia. Furthermore, because similar rates of apoptotic cell engulfment were observed in CX3CR1-deficient and wild-type microglia, it is unlikely that increased apoptotic neurons resulted from inefficient clearance of dead cells by CX3CR1-deficient microglia. Instead, this effect was attributed to reductions in insulin-like growth factor 1 (IGF-1) signaling, a potent trophic factor for NPC survival [22–24], in CX3CR1-deficient mice. In another study, pharmacological inactivation of microglia with minocycline in postnatal rats caused a reduction in the numbers of proliferating progenitor cells and mature oligodendrocytes in the subventircular zone (SVZ). [25] In vitro experiments on cultured neurospheres suggest that this effect is regulated by microglia-derived cytokines including interleukin (IL)-1β, interferon-γ, and IL-6.

While microglia regulate neuronal cell number throughout the brain by initiating NPCD and engulfing dead or dying cells, other work has demonstrated a concomitant function to provide trophic support to progenitor cells (Figure 2, Key Figure). How does a cell simultaneously promote cell death, proliferation, survival, and maturation? Do these functions represent regional differences in neuronal receptivity or heterogeneity of microglia in the brain? Answers to these questions will be important to identify the function of microglia in the developing brain in health and disease.

Regulation of non-neuronal cell development

In addition to neurons, microglia have been implicated in the development of other resident CNS cell types (i.e., other glial cells). For example, in vitro evidence suggests that microglia-conditioned media increases the differentiation of neural stem/precursor cells (NSPCs) into astrocytes through IL-6 and leukaemia inhibitory factor (LIF) [26]. Similarly, microglia-conditioned media promotes survival and differentiation of cultured oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes through a number of secreted factors including IGF-1, nuclear factor kappaB (NF-κB), IL-1β, and IL-6 [25, 27–29]. Microglia have also been suggested to promote myelination by providing iron, a necessary co-factor for myelination, to oligodendrocytes [30–32]. These results suggest that microglia have the potential to regulate survival, proliferation, and maturation of most developing CNS cell types. However, the majority of work assessing the role of microglia in non-neuronal cell development is in vitro. It is unknown whether these mechanisms apply in vivo.

There is also mounting evidence that microglia regulate vascularization of the nervous system. Initial observations showed that microglia arrive in the CNS before blood vessels develop and closely associate with invading vessels, particularly in the developing retina [33,

34]. In vitro and in vivo depletion of microglia in rodents has further suggested that microglia are necessary for vascular branching in the retina and hindbrain [35–38]. However, other studies have reported the opposite effect--depletion of microglia in an ex vivo retinal preparation or in vivo loss of microglial Wnt-ligand transporter (Wntless) expression results in increased vascular branching [39, 40]. Thus, future work is necessary to determine precisely how microglia regulate vasculogenesis. In addition, it remains unknown if microglia-dependent regulation of vascular branching is restricted to the developing retina or whether this is a broader effect occurring throughout the CNS.

Activity-dependent patterning and maturation of neural circuits

Neurons initially connect with each other at synapses to form a crude wiring diagram. Neural activity then regulates the remodeling and maturation of this immature synaptic connectivity whereby less active synaptic connections are eliminated and more active connections are maintained and strengthened [41, 42]. Microglia express neurotransmitter receptors and live imaging studies have revealed that these cells are dynamic sensors of neural activity [43–46]. For example, activity-dependent release of ATP from neurons regulates microglial process motility and outgrowth [47–50] and dampening neural activity int he visual cortex by rearing mice in the dark results in decreased microglial process motility [51]. Furthermore, increasing or decreasing activity in the visual cortex changes the frequency and duration of microglial contact with synapses and induces engulfment of elements that resemble synapses by ultrastructure [51, 52]. These data suggest that microglia could regulate synapse development through activity-dependent mechanisms.

To understand the functional consequences of activity-dependent microglial responses and physical interactions with synapses (Figure 1B–C), recent data has suggested key roles for microglia in regulating maturation and remodeling of synaptic connectivity. Earlier work in acute hippocampal slices prepared from mice mutant for the microglial transmembrane receptor, DNAX-activation protein 12 (DAP12), demonstrated an increase in electrophysiological features characteristic of less mature synapses [53]. In a follow-up study, these DAP12 mutant mice also displayed abnormalities in the development of structural synapses in the hippocampus [54]. In more recent studies, transient reductions in microglia numbers in the hippocampus or barrel cortex due to a genetic deletion of CX3CR1 (CX3CR1 KO) resulted in delayed maturation of structural and functional synapses [55, 56]. These effects are later attenuated in juvenile CX3CR1 KO mice after microglia density reaches wild-type levels. Together, these data suggest that microglia regulate the maturation of synapses in the postnatal brain.

Microglia have also been implicated in the remodeling of developing synapses in response to changes in neural activity. Using the developing mouse retinogeniculate system, a classic model system for studying activity-dependent synaptic remodeling [57–59], microglia were shown to eliminate synaptic connections by engulfing a subset of immature, less active presynaptic inputs [57]. Furthermore, blocking engulfment either pharmacologically or genetically through disruption of complement-mediated phagocytosis resulted in a sustained increase in synapse density and inappropriate connectivity [57, 60, 61]. These data suggest a model by which complement proteins such as C1q and C3 bind or ‘tag’ less active synapses

for removal by microglia via the phagocytic receptor, complement receptor 3 (CR3). This model is supported by in vivo data that C1q and C3 localize to synaptic compartments, synaptic engulfment is reduced in C1q, C3, and CR3-deficient mice, and in vitro data that microglia clear C1q-bound neurites by CR3-dependent phagocytic signaling [60–62]. It remains unknown if and how activity regulates complement proteins. Interestingly, in the context of hypoxic injury and inflammation in the hippocampus, CR3 was necessary to induce long-term synaptic depression (LTD) and suggests microglia can modulate the plasticity of functional synapses via CR3 [63]. Together, these studies demonstrate that microglia respond to changes in neural activity and suggest that they are critical to the remodeling and maturation of synaptic connections in the developing brain.

In addition to modulating development of existing connectivity, microglia have also been implicated in the initial wiring of the embryonic brain. Early work in the developing kitten corpus callosum demonstrated engulfed axonal debris within microglia and astrocytes concomitant with large-scale axonal remodeling [64]. Recent work in the embryonic mouse has demonstrated a similar phenomenon in which microglia appear to engulf a subset of developing tyrosine hydroxylase (TH)-positive, dopaminergic axons [65]. Furthermore, dopaminergic axons were increased at the entrance to the embryonic subpallium in CX3CR1-deficient mice or when mice lacked microglia due to genetic deletion of PU.1 or treatment with an antibody against colony stimulating factor 1 receptor (CSFR1) [65]. Conversely, increasing microglia activation with LPS resulted in a decrease in dopaminergic axons. Interestingly, in addition to dopaminergic axons, interneurons were also affected. Depletion or activation of microglia, as well as genetic deletion of CX3CR1 or DAP12, resulted in premature entry and abnormal distribution of *Lhx6*-expressing interneurons in the embryonic cortical plate and a 10% decrease in a subset of interneurons in the postnatal cortex. In another study, outgrowth and fasciculation of axons within corpus callosum were assessed in embryonic PU.1$^{-/-}$, DAP12$^{-/-}$, or LPS-treated mice [66]. Gene expression profiles at E17.5 revealed a down-regulation of genes related to neuritogenesis in DAP12$^{-/-}$ and LPS-treated mice, which were accompanied by a significant increase in defasciculated axon tracts in the corpus callosum of PU.1$^{-/-}$, DAP12$^{-/-}$, and LPS-treated mice. These studies suggest that impairing microglia during embryogenesis affects axon outgrowth and fasciculation.

In summary, these studies suggest key roles for microglia in the formation and remodeling of neural circuits throughout several regions of the brain (Figure 2, Key Figure). Future work is necessary to elucidate more mechanisms underlying these intercellular interactions and to identify functional consequences. For example, while microglia engulf synapses through the classical complement cascade in the developing visual system, this is likely not the only mechanism. In fact, mammalian astrocytes and *Drosophila* glial cells perform similar functions through different phagocytic receptors including MEGF10 and MERTK in mammals and Draper (the MEGF10 homologue) in *Drosophila* [67–70]. These data raise the question of whether microglia and astrocytes work cooperatively. In addition, it is unknown how mechanisms that regulate microglial responses to changes in neural activity, such as NMDAR-mediated ATP signaling, regulate plasticity and maturation of circuits [47]. It is also unknown whether microglia have a preference for affecting outgrowth, synapse remodeling, and synapse maturation at specific circuits or whether this is a more global

process that occurs throughout the brain. Addressing these questions will be important for understanding the basic biology underlying neural circuit development with tremendous promise for elucidating etiologies of devastating neuropsychiatric disorders with known defects in microglia and brain wiring [2, 71].

Microglia-dependent development of functional brain circuits

Data reveal new roles for microglia in sculpting structural CNS circuitry during development by regulating numbers of cells and synaptic connections as well as regulating spatial patterning of neurons and their projections. Do these functions ultimately translate to the development of functional circuits and appropriate behaviors?

Some of the most compelling evidence that microglia regulate overall circuit function and behavior are experiments in mice in which microglia have been manipulated pharmacologically or genetically. For example, infecting postnatal rats with Escherichia coli followed by a later life immune challenge with LPS resulted in increased hippocampal microglia reactivity and impaired memory in adult mice. [72] Furthermore, 15 min daily handling of postnatal pups (P4-P20) or pharmacological blockade of IL-1β, which is highly expressed by microglia in this context, attenuated these effects [73, 74]. Similarly, immune challenge in a pregnant mouse or nonhuman primate results in offspring with behavioral deficits associated with autism such as changes in ultrasonic vocalizations, abnormal social interactions, and increased repetitive behaviors [75–77]. A similar prenatal immune challenge followed by peripubertal stress in the offspring also resulted in increased microglial reactivity in the pubescent hippocampus and behavioral abnormalities in adult offspring including sensorimotor gating deficits and hypersensitivity to psychotomimetic drugs [78]. In addition, another study manipulated microglia function with minocycline and observed changes in baseline, sex-specific behaviors and synapse architecture [79].

While these studies suggest that microglia can regulate synaptic function, which can ultimately translate to behavior, the pharmacological agents used in these studies are not specific and affect other cells inside and outside the CNS. As a result, other work has taken advantage of powerful molecular genetic approaches to assess the role of microglia in nervous system function. For example, genetic deletion of CX3CR1, a receptor highly enriched in microglia but also expressed by other myeloid-derived cells [22–24], revealed abnormalities in structural connectivity and social behaviors in adult mice [80]. In addition, re-expression of wild-type homeobox B8 (*Hoxb8*) in myeloid derived cells, attenuated pathological grooming behavior in Hoxb8 mutant mice [81]. Similarly, re-expression of methyl CpG binding protein 2 (*Mecp2*) in microglia as well as other myeloid-derived cells attenuated phenotypes in a mouse model of Rett Syndrome (Mecp2 null mice), an X-linked neurodevelopmental disorder [82, 83] (Table 1). However, these data remain controversial [84]. To more specifically manipulate microglia function, two groups recently created mice expressing Cre-ERT2 (Cre recombinase fused to the estrogen receptor for temporal control) under the control of CX3CR1 [85, 86]. This system takes advantage of the relatively high and stable expression of CX3CR1 in microglia and low rate of microglia turnover as compared to other CX3CR1-positive peripheral immune cells [85, 87]. Using this technology, it has been demonstrated that depleting microglia in the juvenile and early adult

CNS using a diphtheria toxin strategy or ablating microglia-derived BDNF results in abnormalities in motor learning [85]. In contrast, depleting microglia using a newly, developed pharmacological strategy, has yielded conflicting results [4]. CSF1R is a cell surface receptor regulating survival, proliferation, and differentiation of microglia and other mononuclear phagocytes [2, 3]. Administration of a drug that inhibits CSF1R (PLX3397) to adult mice results in depletion of primarily microglia with little effect on behavioral measures of anxiety, motor function, learning, or memory [4]. One intriguing notion exists that depletion strongly relies on context. Perhaps, microglia are most critical for establishing brain connectivity and cytoarchitecture necessary for appropriate behaviors in development, a function less critical in the adult. Future work is necessary to identify the relative importance of these cells for overall nervous system function throughout the lifespan of the animal.

While rodents are powerful experimental models that can be used to dissect cellular and molecular mechanisms and assess intermediate phenotypes associated with human neurological disesase, [88–90], there are limits to the system. We are lacking mouse models that closely mimic neurological disease, particularly those that recapitulate the range of behavioral abnormalities associated with psychiatric disorders. Thus, analysis of microglia function in humans is a necessity. Indeed, some of the first evidence suggesting that microglia may play fundamental roles in the functional development of circuits was observed in psychiatric disorders, many of which are now thought to have developmental underpinnings [71, 91, 92]. Early work in postmortem human tissue demonstrated abnormally reactive microglia in brain regions relevant to behaviors associated with a range of psychiatric disorders such as autism, schizophrenia, and bipolar disorder. For example, a study in cerebral cortex demonstrated an increase in MHC class II, human leukocyte antigen-DR (HLA-DR) immunoreactive microglia in autistic versus age-matched control patients [93]. These data suggest that there is increased microglial reactivity in the autistic brain. Since these early studies, we can now map genes to a particular disease. This capability has provided new insight into roles for microglia in nervous system function and has led to the identification of mutations in microglial genes underlying neurological disease [94] (Table 1). Included in these diseases is hereditary diffuse leukoencephalopathy with spheroids (HDLS), an autosomal dominant disease of the CNS white matter caused by mutations in the microglial surface receptor *CSF1R* [95]. Patients suffering from these mutations have demyelination and axonal spheroids accompanied by mood, social, cognitive, and motor impairments. In addition, loss of function mutations in the microglial surface receptors *DAP12* and *TREM2* cause polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL; Nasu-Hakola disease) [96]. This disease is characterized by the development of psychosis and early onset, progressive dementia as well as bone cysts, which are likely due to loss of receptor function in other myeloid-derived cells. Interestingly, while symptoms typically manifest in adulthood in all these disorders, CSF1R, DAP12, and TREM2 are expressed in microglia throughout development. Thus, it is possible that impairments have a developmental underpinning, which become progressively worse and manifest in behavioral changes later in life. In addition to these ‘microgliopathies’, microglia-related genes have recently been identified as risk factors for a number of other neurological diseases including *CD33* and *TREM2* in Alzheimer’s disease,

TREM2 in frontotemporal dementia, *TNFRSF1A* and *IRF8* in multiple sclerosis, and myeloid cell receptor *P2RX7* in bipolar and major depressive disorders [97–104]. In a very recent and exciting study, allelic variations in complement component 4 (C4) in humans were identified as risk factors for developing schizophrenia [105]. Furthermore, human C4 localized to synaptic compartments and mice deficient in C4 had sustained deficits in synaptic remodeling. The authors proposed that similar to C1q and C3, C4 may regulate micorglia-dependent synaptic remodeling. Future work is necessary to determine if these mutations or allelic variations are causative [106].

Concluding Remarks

It is an exciting time to study microglia (see Outstanding Questions). There are now interesting data that microglia can perform a variety of functions in the context of the developing brain including regulating number and maturation of other resident CNS cell types, vascular branching, sculpting synaptic connectivity, regulating axon outgrowth, modulating synaptic maturation and affecting overall behavior (Figure 2, Key Figure). Despite the flurry of new data, many in vitro experiments still require in vivo validation and many studies have used non-specific pharmacological approaches to study microglia function. While recent work has made exciting progress identifying roles for microglia-specific molecules in brain wiring and function, molecular mechanisms are still lacking. Furthermore, data suggest that microglia have separable functions in different brain regions, but elucidating how these regional differences are specified on a cellular and molecular level (i.e., microglial heterogeneity) will be important. Addressing these gaps in knowledge will require new tool development. In particular, there is a need for the identification of more microglia-specific genes that can be used to modulate function as well as the development of strategies to more acutely modulate microglial gene expression in a region-specific manner (e.g. viral-mediated gene delivery). These advancements will have tremendous impact on understanding microglia function in the healthy CNS. Finally, there are a large number of neurological disorders where microglia have now been implicated as central players in disease onset and/or progression [2, 107] (Table 1). However, it is impossible to model the full range of behavioral abnormalities characteristic of human disease in rodents, particularly in the case of psychiatric disorders. Thus, a more sophisticated assessment of microglia function in human patients through functional imaging and gene profiling offers great promise. Identifying molecular mechanisms in the context of animal models and developing technology to assess dysfunction in humans will be critical next steps. These advancements will be necessary to elucidate the basic biology underlying microglia function and for developing diagnostics and therapeutics for devastating neurological disorders with underlying microglia dysfunction.

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- Microglia simultaneously regulate programmed cell death and survival of developing neurons.
- Microglia promote differentiation and provide trophic support for developing astrocytes, oligodendrocytes, and vasculature.
- Formation of neural circuits and activity-dependent remodeling and maturation of synapses are regulated by microglia in the embryonic and postnatal brain.
- Pharmacological and genetic disruption of microglia function results in behavioral abnormalities in juvenile and adult animals.
- Mutations in microglial genes have been identified in human neurological disease including hereditary diffuse leukoencephalopathy with spheroids (HDLS) and Nasu-Hakola disease. In addition, allelic variations in microglia-related genes are associated with increased susceptibility in a number of neurological diseases ranging from Alzheimer's disease to schizophrenia.

Outstanding Questions Box

- Are microglia heterogenous? Microglia regulate an array of functions simultaneously with a high degree of regional specificity. Determining whether microglia are a heterogeneous cell population and identifying how this heterogeneity arises are critical future directions.
- Do microglia regulate the development and maturation of non-neuronal CNS cell types in vivo? The majority of work identifying roles for microglia in regulating the development of non-neuronal cells is in vitro. Thus, it is necessary to determine if the same mechanisms and functions occur in vivo and regulate development throughout multiple regions of the CNS.
- Do microglia work cooperatively with neurons and/or astrocytes to actively initiate synapse remodeling? All data demonstrating that microglia engulf synaptic elements in the developing brain has been in fixed tissue. Therefore, it is unknown whether microglia actively initiate synaptic remodeling and engulf intact synapses or whether they are more passively cleaning up synaptic remnants rendered vulnerable by other neuron or astrocyte-specific mechanisms.
- Do mechanisms regulating neuronal development act in the same pathway or in parallel and are they activity-dependent? Several molecular mechanisms have been identified to regulate microglia-dependent development of neurons and their synaptic connections. However, it is unknown whether these molecular mechanisms work in the same pathway or in parallel or whether these mechanisms are regulated by neural activity.
- Are microglia causative in neurological disorders? Pharmacological and genetic manipulation of microglia has demonstrated changes in behavior in mice and non-human primates. In addition, microglia-related genes have been identified as risk factors for neurological disorders ranging from Alzheimer’s disease to schizophrenia. Whether microglia in the developing brain have roles in disease etiology and behavioral abnormalities remains a mystery.

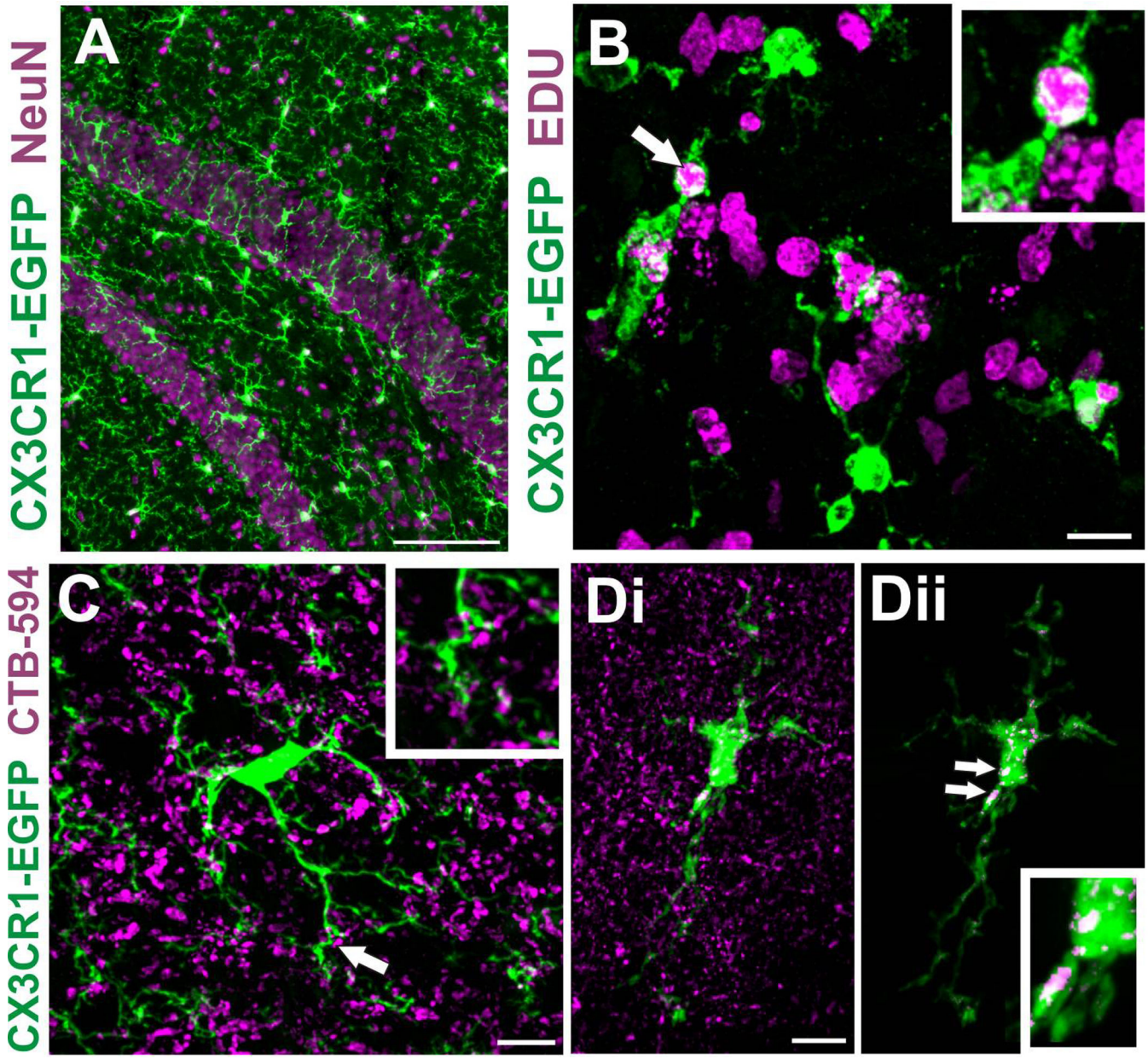

Figure 1. Microglia interact with developing cells in the postnatal brain

A, Microglia (green) in the juvenile (P30) mouse hippocampus represent 5–10% of total CNS cells. Microglia are labeled using a transgenic reporter ($CX3CR1^{egfp/WT}$) and neurons are labeled with an antibody directed against NeuN (purple). Scale bar=100μm. **B**, Microglia ($Cx3CR1^{egfp/WT}$, green) in the SVZ of a P13 mouse engulfing actively dividing cells labeled with 5-ethynyl-2'-deoxyuridine (EDU; purple). Often these apoptotic, dividing cells are found enveloped within microglial processes that form phagocytic cups (arrow and enlarged in inset). **C**, Microglia ($CX3CR1^{egfp/WT}$, green) closely associate and often contact (arrow and inset) retinal ganglion cell (RGC) presynaptic inputs labeled by anterograde tracing with cholera toxin β subunit conjugated to Alexa 594 (CTB-594, purple) in the juvenile mouse lateral geniculate nucleus (LGN, P29). **Di**, Microglia ($CX3CR1^{egfp/WT}$, green) in the early

postnatal LGN (P5) closely associate with RGC presynaptic inputs (CTB-594, purple). **Dii**, Engulfment of presynaptic inputs can be visualized within the microglia soma and processes (arrow, inset) once all RGC input fluorescence outside the microglia volume is subtracted. **B–D**, Scale bar=10μm.

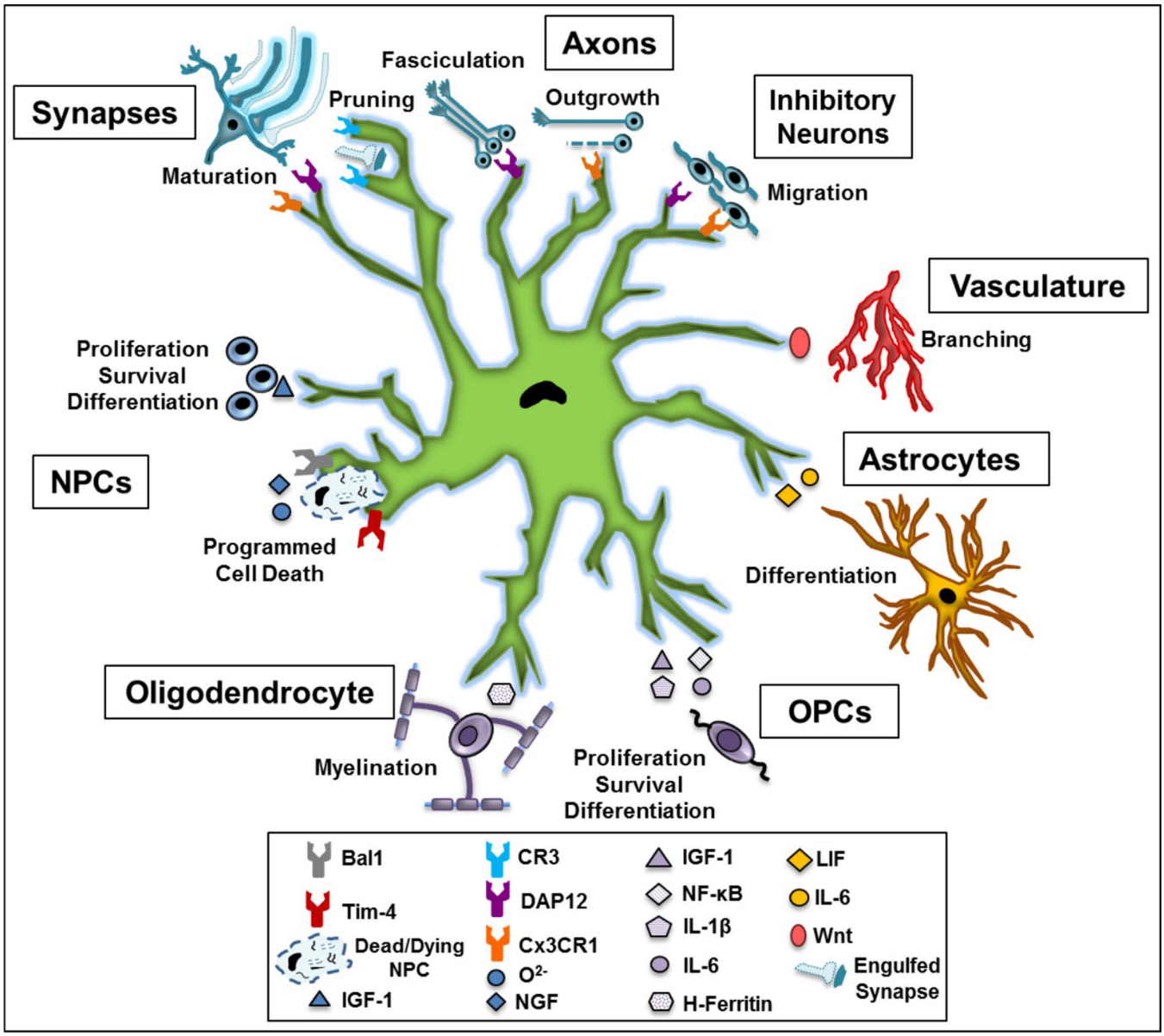

Figure 2. Key Figure. A summary of microglia functions in the developing brain

New data demonstrate that microglia can affect the development of other resident CNS cell types throughout the CNS. NPC, neural precursor cell; OPC, oligodendrocyte precursor cell.

Table 1

Animal models or human diseases associated with microglia dysfunction during development.

Model or Disease	Species	Manipulation	Behavior Affected	Ref
Animal Studies				
Early life infection followed by later life immune challenge	Rat	Prenatal Escherichia coli and later life LPS challenge +/− handling	Memory	[72–74]
Maternal immune activation	Mouse or nonhuman primate	Poly(I:C) in utero +/− postnatal stress	Anxiety, sensorimotor gating, learning, psychotomimetic drug sensitivity, social interactions, ultrasonic vocalizations, repetitive behavior	[75–78]
Estradiol-treated females	Rat	Minocycline	Copulatory behavior	[79]
CX3CR1 KO	Mouse	Gene ablation	Social interactions	[80]
Microglia depletion in juvenile	Mouse	Diphtheria toxin receptor expression using CX3CR1cre-ERT2	Motor learning	[85]
Microglia-specific BDNF null	Mouse	Gene ablation using CX3CR1cre-ERT2	Motor learning	[85]
Hoxb8 mutant	Mouse	Gene mutation +/− wild-type myeloid cells	Grooming	[81]
Mecp2 null	Mouse	Gene mutation +/− wild-type myeloid cells	Locomotion, weight, breathing, and lifespan	[82, 83]
Mecp2 null	Mouse	Gene mutation +/− wild-type myeloid cells	None	[84]
Human Studies				
HDLS	Human	CSF1R mutations	Impairments in mood, social interactions, cognition, and motor control	[95]
Nasu-Hakola disease	Human	*DAP12* or *TREM2* mutations	Psychosis and dementia	[96]
Frontotemporal Dementia	Human	*TREM2* mutation	Dementia	[100]
Alzheimer's disease	Human	*CD33* risk allele; *TREM2* variant	Increased disease susceptibility	[97–99]
Multiple Sclerosis	Human	*IRF8* and *TNFRSF1A* variants	Increased disease susceptibility	[101, 102]
Bipolar and Major Depression	Human	*P2RX7* risk allele	Increased disease susceptibility	[103, 104]
Schizophrenia	human	C4 variant	Increased disease susceptibility	[105]