Cellular and Molecular Life Sciences (2021) 78:3907–3919
https://doi.org/10.1007/s00018-021-03758-1

REVIEW

Mechanisms and significance of microglia–axon interactions in physiological and pathophysiological conditions

Yuki Fujita[1,2] · Toshihide Yamashita[1,2,3,4]

Received: 24 September 2020 / Revised: 28 December 2020 / Accepted: 6 January 2021 / Published online: 28 January 2021

Abstract

Microglia are the resident immune cells of the central nervous system, and are important for cellular processes. In addition to their classical roles in pathophysiological conditions, these immune cells also dynamicallyinteract with neurons and influence their structure and function in physiological conditions. Microglia have been shown to contact neurons at various points, including the dendrites, cell bodies, synapses, and axons, and support various developmental functions, such as neuronal survival, axon elongation, and maturation of the synaptic circuit. This review summarizes the current knowledge regarding the roles of microglia in brain development, with particular emphasis on microglia–axon interactions. We will review recent findings regarding the functions and signaling pathways involved in the reciprocal interactions between microglia and neurons. Moreover, as these interactions are altered in disease and injury conditions, we also discuss the effect and alteration of microglia–axon interactions in disease progression and the potential role of microglia in developmental brain disorders.

Keywords Microglia · Neuron · Axon · Brain · Development · Disease

Introduction

During development, neurons construct neural circuitry through the seamless progression of a discrete series of steps; neurons differentiated from progenitor cells migrate to specific areas, extend their axons toward potential target cells, and connect via synapse formation. These formation events occur in excess; neurons initially form an inordinate number of branches and synapses. Inappropriate connections are removed by axon pruning and/or synapse elimination (Fig. 1). In addition to neuron-intrinsic mechanisms, the interactions of neurons with various cell types, such as glial cells and vascular cells, are also involved in these steps [1, 2]. Recent studies revealed that microglia, which are the resident immune cells of the central nervous system (CNS), play key roles in these processes to establish neural circuits in the developing brain.

Microglia have long been studied for their roles in pathological conditions since they rapidly respond to pathology via changes in their morphology and functions, such as releasing inflammatory cytokines and exhibiting active phagocytic properties [3–5]. More recent studies utilizing new genetic and functional approaches have investigated microglial functions in physiological conditions, particularly in the development of the CNS. Microglia continuously scan the surrounding environment using their processes under physiological conditions [6–9]. Advances in microscopy indicate that microglia contact neurons at various points, such as the cell body, dendrites, axons, and synapses. These interactions are involved in CNS surveillance, and are required for the construction of neural circuitry. In this review, we will provide an overview of neuron–microglia interactions in CNS development, highlighting the signaling and significance of microglia–axon interactions. Moreover, the disturbance of these interactions has been observed in

✉ Yuki Fujita
yuki-fujita@molneu.med.osaka-u.ac.jp

✉ Toshihide Yamashita
yamashita@molneu.med.osaka-u.ac.jp

1 Department of Molecular Neuroscience, Graduate School of Medicine, Osaka University, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan

2 WPI Immunology Frontier Research Center, Osaka University, 3-1, Yamadaoka, Suita, Osaka 565-0871, Japan

3 Graduate School of Frontier Bioscience, Osaka University, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan

4 Department of Neuro-Medical Science, Graduate School of Medicine, Osaka University, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan

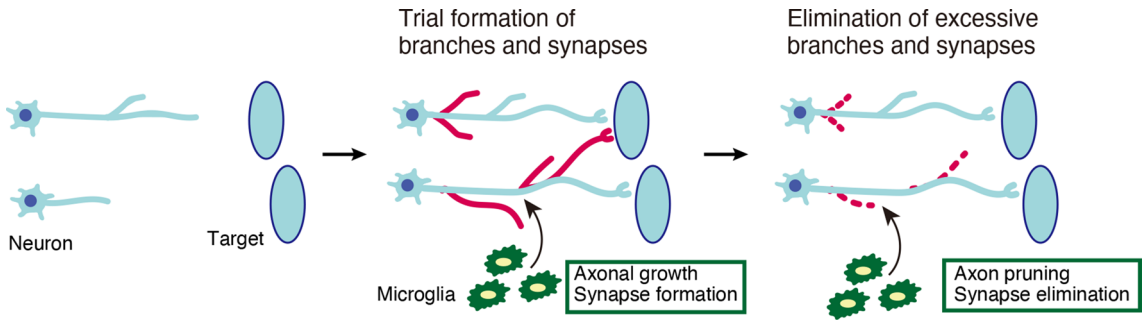

Fig. 1 Schematic model illustrating the refinement of neural circuits and the effect of microglia. Initially, neurons form excessive trial branches and synapses, which are concurrently or sequentially eliminated, resulting in proper network formation

diseases and brain injury conditions, and may contribute to the pathogenesis of neurodevelopmental diseases. Therefore, we will also discuss how microglia–neuron interactions may be affected in disease conditions, and how such knowledge may lead to further advances in the therapeutic strategies for diseases and injuries of the CNS.

The role of microglia in CNS development

Regarding the origin of microglia, del Rio-Hortega demonstrated the concept of a mesodermal origin of microglia based on Cajal's silver carbonate staining [10–12]. There has since been much debate regarding the origin of microglia [13–17]. There are three main theories regarding this point: (1) mesodermal/mesenchymal tissues [18, 19]; (2) the neuroectoderm (similar to neurons, astrocytes, and oligodendrocytes) [20–24]; and (3) hematopoietic cells (circulating blood monocytes), which produce peripheral macrophages [25, 26]. Accumulating evidences support the notion that microglia are derived from myeloid progenitors in the mouse embryonic yolk sac at embryonic day (E) 7.5 [27–30] before infiltrating the brain at E9.5 [13, 27–29, 31, 32]. Microglia then accumulate in clusters close to the white matter, where they actively proliferate. This typical distribution disappears in the adult brain, indicating the specific roles of microglia in the developing brain.

During development, regressive events, such as cell death [33, 34] and synaptic elimination [35–37], are necessary along with constructive events including proliferation, differentiation, axonal elongation, and synaptogenesis to establish proper neural circuitry. Microglia have various functions in these steps. For instance, microglia regulate developmental cell death and survival via their bidirectional functions. Microglia are attracted by apoptotic cells expressing "find-me" signals [38], and "eat-me" signals in the membrane of the apoptotic cell allows microglia to engulf and phagocytose the cell [39]. Uridine diphosphate, the product of uridine-5′-triphosphate hydrolysis, acts on the P2Y6 receptor in microglia to facilitate phagocytosis [40]. Microglia-derived nerve growth factor triggers cell death in the chick retina during early embryonic development [41], suggesting another causal effect of microglia on programmed cell death. In contrast, microglia also express a variety of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), fibroblast growth factor 2, macrophage colony-stimulating factor, osteopontin, and insulin growth factor 1 (IGF-1), which are known to support the proliferation or survival of neurons [42–44].

The details of the various functions and mechanisms of neuron-microglia contact and the history of these topics have been reviewed elsewhere. In the following section, we focus on microglia–axon interactions and discuss how these interactions are coordinated and their role in CNS development.

Axon–microglia interactions in physiological condition

The construction of neural circuits is a dynamic process mediated by both formation and elimination events [35, 45–47]. Initially, neurons form axonal branches and synapses in excessive numbers, and these trial connections are not always maintained in the mature brain. Inappropriate connections are subsequently or concurrently removed. In the following section, we summarize the microglial functions in these constructive and deconstructive processes, and discuss how they affect neural circuit formation (Fig. 1). Similar to other fundamental developmental processes, such as cell death/survival, and synapse formation/elimination, microglia also exert both constructive and deconstructive effects on axons with a certain amount of common molecular mechanisms.

Microglia–axon interactions support neuronal survival and early network wiring

In the developing brains of various species, including humans, microglia exhibit a specific and heterogeneous distribution [48–51]. In particular, microglia accumulate at a

higher density at the developing axonal tract during a specific period of brain development, and these microglia demonstrate a unique morphology (Fig. 2) [52–54]. The accumulation of microglia around axons was initially described in the early 1900s [10, 11] and is now widely recognized in both rodents and humans [48–51, 55–57]. Further, in vivo two-photon imaging studies provided evidence that microglia can directly contact neurons [58].

An early study using primary culture systems reported that conditioned media from primary microglia cultures isolated from embryonic rat brains enhances neurite outgrowth [59]. Blockade of the extracellular matrix protein thrombospondin by antibodies inhibits this effect, suggesting that microglia mediate neurite growth by releasing thrombospondin. Conditioned media from microglial cultures also increase the proliferation of cultured cerebellar granule cells via mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt, and delta-Notch signaling pathways [60]. These findings suggest that embryonic microglia support the processes of neural circuit formation.

The accumulation of microglia is notably observed in dopaminergic axons during the embryonic stage (Fig. 2a) [52]. Microglial depletion or dysfunction via disruption of microglial signaling or maternal immune activation (MIA) modulates the outgrowth of dopaminergic axons. Microglial depletion via colony-stimulating factor 1 receptor (CSF-1R) blockade in Cx3cr1 green fluorescent protein (GFP)/+ or Pu.1 −/− mutants results in the exuberant extension of tyrosine hydroxylase (TH)-positive dopaminergic axons into the subpallium of the embryonic ventral telencephalon, whereas MIA induces a mild but robust reduction in the extension of TH-positive axons into the subpallium at E14.5. Three-dimensional reconstruction of confocal images and electron microscopy demonstrated direct contact between microglia and TH-positive axons, and TH-positive axon fragments were detected inside the cytoplasms of GFP-positive microglia. These findings suggest that microglia might phagocytose fragments of dopaminergic axons in physiological conditions. Interestingly, no defects in the neighboring serotoninergic fibers or internal capsule were observed, suggesting that microglial regulation of axon outgrowth is rather specific to dopaminergic fibers in the embryonic brain.

Microglia also contact developing axonal fibers in the corpus callosum and mediate its fasciculation in the embryonic brain (Fig. 2b). The loss of DAP12, a key microglial-specific signaling molecule; depletion of microglia in Pu1 −/− mice; and an MIA model demonstrated the defasciculation of dorsal callosal axons as detected by the increased

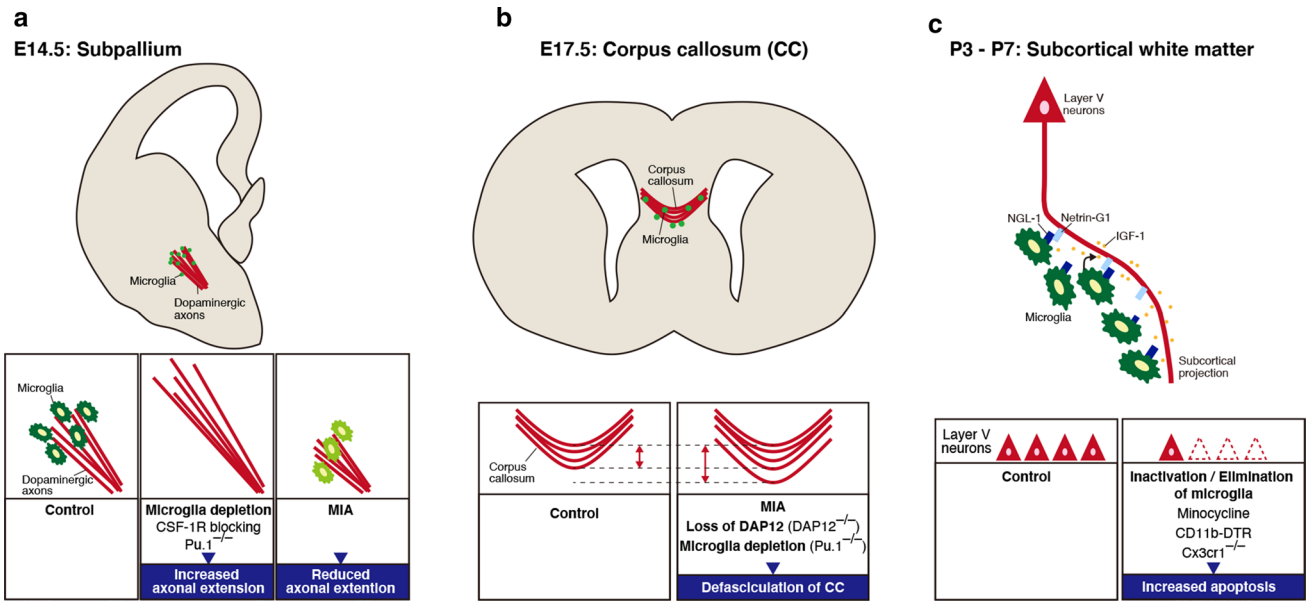

Fig. 2 Various interactions between microglia and axons during brain development. **a** Microglia (green) accumulate around dopaminergic axons (red) in the subpallium at embryonic day (E)14.5. Pharmacological [colony-stimulating factor 1 receptor (CSF-1R) blocking] or genetic (Pu.1 −/− mutants) depletion of microglia promotes dopaminergic axonal outgrowth into the striatum, whereas maternal immune activation (MIA) reduces the extension of dopaminergic axons. **b** Microglia accumulate around axonal fibers in the corpus callosum (CC) and mediate its fasciculation at E17.5 via a mechanism involving DAP12 signaling. The loss of DAP12, depletion of microglia in Pu1 −/− mice, and MIA lead to defasciculation of the CC. **c** Microglia accumulate around the axons from layer V neurons, peaking at postnatal day (P)3–7, and support the survival of layer V neurons via the release of insulin-like growth factor 1 (IGF-1). Pharmacological inactivation (minocycline treatment), and genetic inactivation or elimination of microglia (Cx3cr1 −/− mouse or CD11b-DTR transgenic mice) increases the rate of apoptosis in the layer V neurons. NetirnG1 expressed in neurons and its ligand netrin-G ligand-1 (NGL1) expressed in microglia are involved in this interaction

ratio of neuropilin 1-positive dorsal tract width normalized to the width of L1-CAM-positive callosal fibers at E17.5 [54]. Remarkably, the time-course of microglial accumulation assessed in this study slightly differed from that in dopaminergic axons [52].

In the early postnatal brain, microglia accumulate around the axons of layer V neurons and support the survival of these neurons (Fig. 2c) [53]. Microglia begin to accumulate in the white matter, including the subcortical white matter, internal capsule, cerebral peduncle, and cerebellar white matter, where the axons of corticospinal motor neurons in layer V pass, from postnatal day (P)1–3. Their levels peaked in these areas at P3–7. Apoptosis of layer V neurons was observed in the three different models involving the inhibition of microglial activation with minocycline and transient genetic depletion of microglia with Cd11b-DTR mice and CX3CR1 knockout mice. Both in vitro and in vivo observations demonstrate that this effect of microglia on neuronal survival is due to the release of IGF-1, which enhances axon outgrowth of corticospinal motor neurons [61]. Further, microglial accumulation at this axonal tract is mediated by netrin-G1-netrin-G ligand-1 (NGL1) signaling in the postnatal brain [62]. Netrin-G1 is specifically expressed in corticospinal neurons and at high levels in the postnatal period [63]. Netrin-G1 is structurally related to the netrin family of axon guidance molecules [64–66]. However, unlike classic netrins, it is linked to the plasma membrane surface by a glycosylphosphatidylinositol anchor and lacks affinity for the known netrin receptors, DCC and UNC-5. Instead, netrin-G1 binds to a cell adhesion molecule, NGL1. Deletion of netrin-G1 or NGL1 expression reduces the accumulation of microglia along the axons of layer V neurons.

The inactivation of microglia by minocycline treatment from P3 to P4 induces the death of cortical neurons [53]. However, an increased rate of apoptosis was not observed in layer V or other layers when minocycline was administered to mice at E16–17 or P14–15 when amoeboid microglia were not clustered in the white matter. Consistently, another group reported that the death of layer V neurons in the somatosensory cortex was not observed when mice were treated with minocycline from P8 to P10 [67]. These observations suggest that the time-point of the effect of microglial inhibition is finely restricted.

Collectively, microglia accumulate at different axons at different time-windows during development, and play a key role in supporting axons and neurons. The organization of the dynamic alteration of temporal and spatial microglial accumulation and the link to the modulation of certain axonal tracts would be clarified in the future studies.

Axons in the CNS can be supported by myelin sheaths created by oligodendrocytes, which may also be associated with the accumulation of microglia along axons. Indeed, crosstalk between microglia and oligodendrocytes has been noted in both physiological and pathological conditions [68–70]. Similar to how neurons construct excess axons and synaptic networks and are selectively eliminated, myelin sheaths are first overproduced and pruned to refine myelination during development [71]. A study using in vivo imaging of zebrafish has revealed that microglia phagocytose myelin sheaths lead to myelin refinement [68]. Neuronal activity attracts microglia towards neuronal cell bodies, whereas less neuronal activity promotes microglia to associate with axons and phagocytose myelin. Thus, microglia–axon interactions and myelin phagocytosis are bidirectionally regulated by neuronal activity.

Effects on axon pruning

The excess branches formed by unconstrained growth of axons in the initial phase are pruned in a degenerative or retractive manner [46, 47, 72]. The impairment of axon pruning leads to behavior deficits [73, 74], indicating that this deconstruction process is also critical for CNS development as well as the processes involved in the early wiring of the CNS, such as axon outgrowth.

Axon pruning in drosophila mushroom body γ neurons has been widely used as a model to assess the mechanism of pruning, since it occurs in a defined set of temporally-regulated morphological changes, hallmarked by the initial extension of excess axons during the larval stage, and following fragmentation of axons between 8 and 12 h after puparium formation [75, 76].

In this model, glial cells surrounding the axon processes engulf degenerating axons [77, 78]. Interestingly, the invasion of glia at these axons precedes axon fragmentation, suggesting that glial invasion occurs rather independently of activation in response to axon fragmentation [77]. These findings indicate that glia may play an initiative role in triggering axon fragmentation. The glial proteins draper and ced-6, which are essential for the clearance of apoptotic cells in *C. elegans*, are required in the glial engulfment of axons in the larval stage [79]. Since ecdysone signaling, which mediates a cell-autonomous genetic program for axon pruning [80, 81], induces draper expression, this signaling may link the intrinsic and extrinsic programs for axon pruning during *Drosophila* metamorphosis [79].

Although it should be noted that these processes could involve neuroectodermally derived cells with immune functions that are different from microglia cells and but more like macroglial cells, their ability to engulf degenerating neurons reflects the phagocytic aspect of microglia in mammals. Microglia engulf axon debris via the p38 mitogen-activated protein kinase pathway in the axons of rodent cortical explants [82]. However, the role and underlying mechanisms of microglia in axon pruning remain unclear. The mechanism underlying the clearance of axon debris is more evident

in pathological conditions, which is described in the following Sect. ("Alteration of microglia–axon interactions in the pathological conditions").

Interaction of microglia with the axon initial segment

In addition to these diverse interactions of microglia and axons, microglia preferentially interact with the axon initial segment (AIS) located at the base of axons, which is responsible for action potential generation and is a major regulator of neuronal excitability [83]. A subset of microglia that are intimately associated with the neuronal cell body extend a single process and contact the AIS [84]. Microglia–AIS interactions occur early in the postnatal stage (from P9) and are maintained throughout adulthood (8–12 weeks in mice).

Further, these interactions exhibit specificity with regard to brain regions. AIS-associated microglia are more prominently observed in the cortex than in the thalamus and striatum. Furthermore, AIS-associated microglia preferentially associate with non-GABAergic neurons than GABAergic neurons, and more frequently associate with pyramidal neurons in layer V of the cortex compared to the total cortical neurons, suggesting that AIS-associated microglia favor excitatory neurons. Neuron-specific knockout of ankyrin G reduced the number of neurons with AIS-associated microglia, suggesting that, although microglia can contact various parts of neurons, a certain subset of microglia may recognize the AIS via ankyrin G expression. Since microglia–AIS interactions are lost following microglial activation 3–72 h after traumatic brain injury, these interactions may be more conspicuously involved in controlling healthy brain functions than in modulating response to injury. Although the function of AIS-associated microglia remains unclear, the anatomical and physiological properties of the AIS suggest that those microglia may be to modulate the excitable properties of the AISs they contact or monitor AIS synapses.

The role of microglia in the refinement of synaptic circuits

To establish appropriate synaptic connections, neural circuits are refined and reinforced by the removal of exuberant synapses. There has been increasing interest in the role of microglia in synaptic refinement, particularly regarding the activity-dependent mechanism [9, 85]. In line with neural network formation, we briefly summarize the functions of interactions between microglia and synapses.

As well as intrinsic programs, extrinsic cues resulting in neural activity are critical for the proper maturation and maintenance of the neural network [86]. In vivo two-photon imaging has been used to determine the role of microglia in the regulation of neuronal excitability. Resting microglia in the mouse visual cortex can contact dendritic spines via their dynamic processes [87, 88]. Microglial processes make brief and direct contacts with neuronal synapses within minutes at a frequency of approximately one per hour. [88]. Global downregulation of neural activity following the injection of the voltage-gated sodium channel blocker tetrodotoxin (TTX) reduced the frequency of such contacts, indicating that microglia-synapse contacts are activity-dependent in physiological conditions. During alterations in visual experience, microglial processes changed their morphology and microglial behavior was altered, such as increased phagocytosis of synaptic elements and interaction with subsets of structurally dynamic and transient synapses, suggesting that microglia contribute to the activity-dependent modification or elimination of synapses for experience-dependent plasticity in the healthy brain [87]. In vivo time-lapse imaging of both microglial morphology and neuronal activity in zebrafish larvae indicated the reciprocal regulation of microglia and neuronal activity [89]. Neuronal activity induces resting microglia to make contact with active neurons, resulting in the selective reduction of contacted neuron activity.

When the repetitive stimulation of individual layer II/III pyramidal neurons induces neuronal hyperexcitability and elicits swelling of axons, microglial processes migrate to and wrap these damaged axons following the release of both ATP, known to be a potent attractant of microglia, and glutamate via volume-activated anion channels (VAACs) [90]. This microglia–axon contact induces rapid repolarization in the neuron, which reverses the hyperexcitability to resting levels. Inhibition of microglial migration by pharmacological blockage of VAACs sustains the activity-induced depolarization until cell death, indicating that the microglia–axon interaction contributes to the protection of the neuronal soma from damage due to pathological activity and to the maintenance of neuronal viability.

Taken together, these observations suggest that microglia can control the proper window for neuronal activity, which is essential for the maturation and maintenance of neural circuitry. Therefore, they contribute to brain functions, such as learning and memory, in the healthy brain.

Microglia eliminate synapses through interactions and phagocytosis via signaling pathways involving the chemokine fractalkine (Cx3cl1) and its receptor, Cx3cr1 [92, 93], and complement pathways [91], which are involved in microglial migration and phagocytic functions, respectively.

Stimulated emission depletion microscopy and a combination of electron microscopy with immunolabeling can detect both pre- and post-components of excitatory neurons in the cytoplasm of microglial cells in the hippocampus, indicating the engulfment of synaptic materials by microglia [92]. Cx3cr1-knockout mice showed a transient reduction

in the number of microglia and an increase in the number of dendritic spines. The impairment of synaptic elimination in these mice leads to weak synaptic transmission, reduced functional brain connectivity, and behavior deficits associated with neurodevelopmental and neuropsychiatric disorders [93].

Intensive studies revealed that neural activity modulates elimination processes during CNS development [94–96]. The retino-genicular circuit system is a classical model for studying activity-dependent synaptic elimination [97–99]. Complement component C3 expressed in immature synapses and its receptor complement receptor 3 (CR3/CD11b-CD18/macrophage antigen-1) expressed on the surface of microglia are necessary for the elimination of retino-geniculate synapses at the postnatal stage [91, 100]. Blockade of retinal ganglion cell (RGC) activity by TTX increased the engulfment of its connection by microglia, whereas increased RGC activity by forskolin reduced the engulfment of its connection by microglia. These observations suggest that microglia contribute to the elimination of the retino-genicular connection, preferentially at less active (weaker) synapses.

In addition to elimination, microglia are also involved in synapse formation via direct contact with dendrites in layer II/III pyramidal neurons in the developing somatosensory cortex at P8-10 when intense synaptogenesis occurs [101]. Microglia contact with dendrites induces filopodia formation, which typically occurs at dendrites with contact-induced Ca^{2+} elevation.

Depletion of microglia impaired the turnover of spines on pyramidal neurons of the motor cortex, with an effect on both the elimination and the formation of spines in the layer V pyramidal neurons of late postnatal (P19) and young adult (P30) mice [102]. Microglia-derived BDNF is a key mediator of this synaptic plasticity since microglia-specific knockout of BDNF largely recapitulated the altered structural plasticity in the spines and performance deficits in microglia-depleted mice.

Alteration of microglia–axon interactions in the pathological conditions

Following neural insults or diseases, the pathological state is progressively altered [103]. Axonal damage causes axon degeneration and generates myelin debris derived from broken myelin sheaths, which contain myelin-derived inhibitors to axon growth [104, 105]. This debris not only constitutes obstructions and inhibitory extrinsic cues for axonal regrowth/regeneration, but also triggers undesirable inflammation, leading to further damage to axons and neurological dysfunction [106, 107].

Following pathological events, such as injury, inflammation, infection, and neurodegeneration, microglia quickly respond and can accumulate at the lesion site accompanied by morphologic alteration from a resting state referred to as "ramified" to an active state. Microglia are involved in neurotrophic support and the removal of damaged cells and debris, which is important for reconstructing the neural network (Fig. 3) [108, 109]. The following section discusses the bidirectional functions of the microglia–axon interaction in favor of and in opposition to the reconstruction of neural circuits.

Inflammation

In physiological conditions, microglia continuously monitor the brain environment, but are prominently activated in response to neural insult. Abnormal microglial activation contributes to secondary damage to neural circuits [110–115]. Reactive microglia abundantly express or release various inflammatory proteins, such as tumor necrosis factor-α, inducible nitric oxide synthase, or interleukin-1, which leads to the production of nitric oxide and reactive oxygen species (Fig. 3a) [116–118].

However, the activation of microglia might be important as it facilitates their phagocytic activity, which arranges

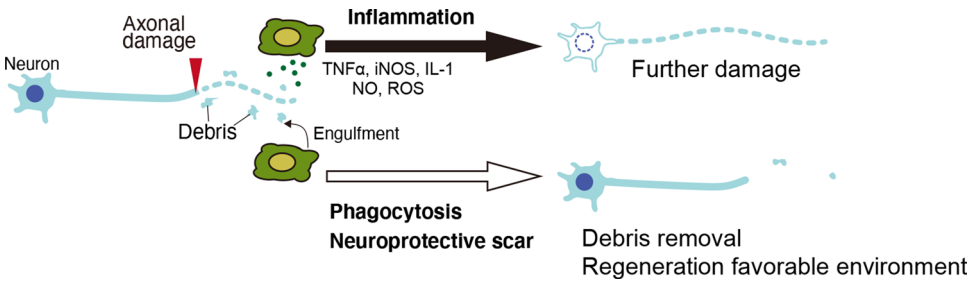

Fig. 3 Schematic representation of the role of microglia–axon interactions in pathological conditions. **a** Microglia have both beneficial and detrimental effects in disease and injury, and are involved in inflammation following axonal damage. **b** The active phagocytic activity of microglia following axonal damage contributes to debris clearance and arranges the environment into a favorable condition for regeneration. *TNF-α* tumor necrosis factor-α, *iNOS* inducible nitric oxide synthase, *IL-1* interleukin-1, *NO* nitric oxide, *ROS* reactive oxygen species

the environment into a favorable condition for regeneration, such as the removal of myelin debris or damaged cells (Fig. 3b), and proper stripping of the synapse to protect neurons from excessive neural excitation. Regardless, the excessive or continuous activation of microglia could shift the microglia into a detrimental-dominant role, and contribute to acute and chronic neuropathology. This is evidenced by a mouse model of Alzheimer's disease (AD). TDP-43 is a DNA–RNA binding protein that regulates microglial phagocytosis in the Aβ42 oligomers-injected AD model mouse [119]. Mice lacking TDP-43 in microglia show enhanced phagocytosis in AD model mouse, but at the same time, exacerbate synaptic loss even in the absence of amyloid. These observations indicate the causative role of microglia in the pathogenesis of neurodegenerative disorders, and suggest that controlling phagocytic activity is a good strategy to regulate disease progression.

Debris clearance to allow a favorable environment for axon regeneration

In rat ischemia models, activated microglia /macrophages rapidly migrated to the infarct area and displayed phagocytic features at day 1 after ischemia [120, 121]. Resident microglia, rather than in filtrating macrophages, likely contribute to debris clearance in the transient focal cerebral ischemia model induced by middle cerebral artery occlusion using GFP-transgenic bone marrow chimeric mice [122].

In a dog spinal cord injury (SCI) model, the number of microglia with a phagocytic phenotype increased in the later stage of injury. However, these microglia exhibited reduced phagocytic activity [123].

In a zebrafish traumatic brain injury model, microglia rapidly accumulated at the lesion site and removed damaged cells by phagocytosis after the injury. In this model, two distinct phases of cell death occur: primary cell death as detected by propidium iodide (PI)-positive cells peaked immediately after injury, and secondary cell death as detected by pyknotic nuclei peaked at 6 h post injury. Live imaging of *p2y12*:GFP;*mpeg1*:mCherry double transgenic zebrafish larvae revealed that p2y12+/mpeg1+ microglia rather than p2y12−/mpeg1+ macrophages infiltrated the brain at the lesion site after the injury. Microglia rapidly phagocytosed PI-positive cells 0.5 h after brain injury. The pharmacological inhibition of phosphatidylserine-dependent phagocytosis by *O*-phospho-L-serine or CRISPR/Cas9-mediated gene knockdown of microglial phagocytosis by injection of gRNA targeting adgrb1a and adgrb1b [124] suppressed the engulfment of PI-positive damaged cells. Remarkably, this increased secondary cell death 6 h after injury, indicating that the rapid clearance of cellular debris by microglial phagocytosis reduces secondary cell death after brain injury.

These findings suggest that the rapid phagocytic activity of microglia following injury largely contributes to the removal of damaged axons, which is beneficial to induce efficient reconstruction of neural circuits.

Accumulating studies demonstrate that regressive events are also involved in the progressive process of neural circuit reconstruction following injury [125–127]. In rodent SCI models, injured corticospinal tract axons above the injury site form a large number of new collaterals and synaptic contacts to interneurons in the spinal cord at the initial phase. The numbers of these connections are reduced in the later phase that contribute to the refinement and reinforcement of a detour circuit to the below target area that avoids the lesion site, leading to functional recovery. These processes following axonal damage are similar to the formation and deconstruction processes during de novo construction of neural circuits at the developmental stage. Although there is no solid evidence that microglia are involved in these processes, accumulating evidences on their active phagocytic abilities following neural injury or diseases suggest the potential role of microglia in such processes.

A recent study revealed that microglia are involved in scar-free wound healing after crush injuries to the spinal cord in neonatal mice [128]. Similar to fish and amphibians, neonatal mice are capable of scar-free wound healing and spontaneous axon regrowth, although this is not the case in adult mammals [129]. Single-cell RNA-seq analysis revealed the features of repair-promoting microglia populations with a significant enrichment for genes related to the extracellular matrix. Transplantation of neonatal microglia or proteinase inhibitor treatment help improve wound healing and axon regeneration after SCI in adult mice. Thus, neonatal microglia can have a protective function even in the adult injury, and it would be intriguing to develop a potential application that enables a shift in the microglia to its neonatal state during disease conditions.

Additional roles of microglia in pathophysiological conditions.

In line with the importance of microglial phagocytosis in normal brain development, disruption of this function is also linked to neurodevelopmental diseases. Microglia from MECP2-null mice, a mouse model of Rett syndrome, exhibited reduced phagocytic activity and replacement with wild-type microglia arrested disease progression (Derecki NC, 2012, Nature). Thus, the phagocytic properties of microglia are indispensable for normal brain development and function, and deficits in phagocytosis might be associated with neurodevelopmental diseases.

In addition to their phagocytic activity, microglia can support neurons via mechanisms involving the release of neuroprotective factors [130–132]. In a mouse SCI model,

microglia induced astrocyte proliferation via IGF-1 and supported neuroprotective scar formation. Depletion of microglia after the injury caused disruption of glial scar formation, enhanced immune infiltration, exacerbated axonal damage, and impaired locomotor recovery [132, 133].

Thus, reactive microglia can also have beneficial effects in pathological conditions via phagocytosis and the production of factors associated with neuroprotective functions.

Conclusion and perspectives

The diverse functions of microglia have further been demonstrated in physiological and pathological conditions. As featured in this review, microglia–axon interactions have long been known to elicit various effects in the construction or reconstruction of neural circuits during development and following injury and diseases. The molecular mechanisms of these interactions have been progressively unveiled. In the rapidly expanding research area on the interaction between the nervous system and the immune system in the CNS, more questions have been raised.

For instance, how microglia communicate with axons remains far from clear. Microglia distribute ubiquitously in the brain, but accumulate at specific axons at high levels, and this preferential association can be altered in a fine time-window of development. One prominent limitation that makes it difficult to analyze these spatio-temporal specific interactions is the challenge of tracking both microglia and developing axons in the wide field of brain with both spatial and temporal information. Advances in the techniques used to generate genetically modified animal models expressing cell type-specific and inducible reporters, and in vivo imaging analysis will certainly help us to surpass these limitations.

In addition, although remarkable progress has been made toward understanding the molecular mechanisms underlying microglia–axon interactions, we need to understand how best to apply these findings to develop novel diagnostic and therapeutic strategies for diseases and injuries of the CNS. Interestingly, microglia have the potential to alter their identity even in the adult brain by forced expression of a single transcription factor, NeuroD1. NeuroD1 is an essential transcription factor for the generation of neurons in the developing brain, and can directly converts mouse microglia into induced neuronal cells that express mature neuronal markers and form synaptic networks with primary cultured cortical neurons [134]. These induced neuronal cells are accompanied by global remodeling of the microglial epigenetic signature. Furthermore, the induced neuronal cells were functionally integrated into brain circuits via synaptic connections with other neurons in the adult mouse brain. Strategies for reprogramming pathological microglia that accumulate at lesion sites into neurons or inducing the neuroprotective character would be beneficial for the development of novel therapeutic approaches for nerve injury and disease.

This is the era that enables us to address the molecular features of cells at the single-cell level [135–139] Microglia demonstrate greater diversity during development and disease or in the aging brain than in the normal, healthy adult brain [135]. To specify microglial heterogeneity and decipher how transcription is regulated in such unique developmental microglia–axon interactions would be of great benefit in answering these fundamental questions regarding development and disease and in the development of novel therapeutic approaches.

Author contributions YF performed the literature search and drafted the manuscript. TY had the idea for the article and revised the work.

Funding Not applicable.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. Catapano LA, Arnold MW, Perez FA, Macklis JD (2001) Specific neurotrophic factors support the survival of cortical projection neurons at distinct stages of development. J Neurosci 21(22):8863–8872
2. Dugas JC, Mandemakers W, Rogers M, Ibrahim A, Daneman R, Barres BA (2008) A novel purification method for CNS projection neurons leads to the identification of brain vascular cells as a source of trophic support for corticospinal motor neurons. J Neurosci 28(33):8294–8305. https://doi.org/10.1523/JNEUROSCI.2010-08.2008
3. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiol Rev 91(2):461–553. https://doi.org/10.1152/physrev.00011.2010
4. Ransohoff RM, Perry VH (2009) Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol 27:119–145. https://doi.org/10.1146/annurev.immunol.021908.132528
5. Shemer A, Erny D, Jung S, Prinz M (2015) Microglia plasticity during health and disease: an immunological perspective. Trends Immunol 36(10):614–624. https://doi.org/10.1016/j.it.2015.08.003
6. Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387–1394. https://doi.org/10.1038/nn1997
7. Ueno M, Yamashita T (2014) Bidirectional tuning of microglia in the developing brain: from neurogenesis to neural circuit formation. Curr Opin Neurobiol 27:8–15. https://doi.org/10.1016/j.conb.2014.02.004
8. Mosser CA, Baptista S, Arnoux I, Audinat E (2017) Microglia in CNS development: Shaping the brain for the future. Prog Neurobiol 149–150:1–20. https://doi.org/10.1016/j.pneurobio.2017.01.002

9. Wake H, Moorhouse AJ, Miyamoto A, Nabekura J (2013) Microglia: actively surveying and shaping neuronal circuit structure and function. Trends Neurosci 36(4):209–217. https://doi.org/10.1016/j.tins.2012.11.007
10. del Rio-Hortega P (1919) El "tercer elemento" de los centros nerviosus. Bol Soc Esp Biol 9:69–120
11. del Rio-Hortega P (1932) Microglia, vol 2. Cytology and cellular pathology of the nervous system. Hoeber, New York
12. Sierra A, de Castro F, Del Rio-Hortega J, Rafael Iglesias-Rozas J, Garrosa M, Kettenmann H (2016) The "Big-Bang" for modern glial biology: translation and comments on Pio del Rio-Hortega 1919 series of papers on microglia. Glia 64(11):1801–1840. https://doi.org/10.1002/glia.23046
13. Chan WY, Kohsaka S, Rezaie P (2007) The origin and cell lineage of microglia: new concepts. Brain Res Rev 53(2):344–354. https://doi.org/10.1016/j.brainresrev.2006.11.002
14. Rezaie P, Male D (2002) Mesoglia and microglia—a historical review of the concept of mononuclear phagocytes within the central nervous system. J Hist Neurosci 11(4):325–374. https://doi.org/10.1076/jhin.11.4.325.8531
15. Ginhoux F, Lim S, Hoeffel G, Low D, Huber T (2013) Origin and differentiation of microglia. Front Cell Neurosci 7:45. https://doi.org/10.3389/fncel.2013.00045
16. Bertrand JY, Jalil A, Klaine M, Jung S, Cumano A, Godin I (2005) Three pathways to mature macrophages in the early mouse yolk sac. Blood 106(9):3004–3011. https://doi.org/10.1182/blood-2005-02-0461
17. Palis J, Robertson S, Kennedy M, Wall C, Keller G (1999) Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development 126(22):5073–5084
18. Boya J, Calvo J, Prado A (1979) The origin of microglial cells. J Anat 129(Pt 1):177–186
19. Ashwell K (1991) The distribution of microglia and cell death in the fetal rat forebrain. Brain Res Dev Brain Res 58(1):1–12
20. Fujita S, Tsuchihashi Y, Kitamura T (1981) Origin, morphology and function of the microglia. Prog Clin Biol Res 59A:141–169
21. Paterson JA, Privat A, Ling EA, Leblond CP (1973) Investigation of glial cells in semithin sections. 3. Transformation of subependymal cells into glial cells, as shown by radioautography after 3 H-thymidine injection into the lateral ventricle of the brain of young rats. J Comp Neurol 149(1):83–102. https://doi.org/10.1002/cne.901490106
22. de Groot CJ, Huppes W, Sminia T, Kraal G, Dijkstra CD (1992) Determination of the origin and nature of brain macrophages and microglial cells in mouse central nervous system, using non-radioactive in situ hybridization and immunoperoxidase techniques. Glia 6(4):301–309. https://doi.org/10.1002/glia.440060408
23. Fedoroff S, Zhai R, Novak JP (1997) Microglia and astroglia have a common progenitor cell. J Neurosci Res 50(3):477–486. https://doi.org/10.1002/(SICI)1097-4547(19971101)50:3%3c477::AID-JNR14%3e3.0.CO;2-3
24. Hao C, Richardson A, Fedoroff S (1991) Macrophage-like cells originate from neuroepithelium in culture: characterization and properties of the macrophage-like cells. Int J Dev Neurosci 9(1):1–14. https://doi.org/10.1016/0736-5748(91)90067-v
25. Kaur C, Ling EA, Wong WC (1987) Origin and fate of neural macrophages in a stab wound of the brain of the young rat. J Anat 154:215–227
26. Ling EA (1994) Monocytic origin of ramified microglia in the corpus callosum in postnatal rat. Neuropathol Appl Neurobiol 20(2):182–183
27. Alliot F, Godin I, Pessac B (1999) Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain Res Dev Brain Res 117(2):145–152
28. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330(6005):841–845. https://doi.org/10.1126/science.1194637
29. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW, Frampton J, Liu KJ, Geissmann F (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336(6077):86–90. https://doi.org/10.1126/science.1219179
30. Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, Wieghofer P, Heinrich A, Riemke P, Holscher C, Muller DN, Luckow B, Brocker T, Debowski K, Fritz G, Opdenakker G, Diefenbach A, Biber K, Heikenwalder M, Geissmann F, Rosenbauer F, Prinz M (2013) Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat Neurosci 16(3):273–280. https://doi.org/10.1038/nn.3318
31. Chen SK, Tvrdik P, Peden E, Cho S, Wu S, Spangrude G, Capecchi MR (2010) Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. Cell 141(5):775–785. https://doi.org/10.1016/j.cell.2010.03.055
32. Herbomel P, Thisse B, Thisse C (2001) Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. Dev Biol 238(2):274–288. https://doi.org/10.1006/dbio.2001.0393
33. Oppenheim RW (1991) Cell death during development of the nervous system. Annu Rev Neurosci 14:453–501. https://doi.org/10.1146/annurev.ne.14.030191.002321
34. Yeo W, Gautier J (2004) Early neural cell death: dying to become neurons. Dev Biol 274(2):233–244. https://doi.org/10.1016/j.ydbio.2004.07.026
35. Lichtman JW, Colman H (2000) Synapse elimination and indelible memory. Neuron 25(2):269–278. https://doi.org/10.1016/s0896-6273(00)80893-4
36. Chung WS, Barres BA (2012) The role of glial cells in synapse elimination. Curr Opin Neurobiol 22(3):438–445. https://doi.org/10.1016/j.conb.2011.10.003
37. Wilton DK, Dissing-Olesen L, Stevens B (2019) Neuron-glia signaling in synapse elimination. Annu Rev Neurosci 42:107–127. https://doi.org/10.1146/annurev-neuro-070918-050306
38. Xu J, Wang T, Wu Y, Jin W, Wen Z (2016) Microglia colonization of developing zebrafish midbrain is promoted by apoptotic neuron and lysophosphatidylcholine. Dev Cell 38(2):214–222. https://doi.org/10.1016/j.devcel.2016.06.018
39. Sierra A, Abiega O, Shahraz A, Neumann H (2013) Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. Front Cell Neurosci 7:6. https://doi.org/10.3389/fncel.2013.00006
40. Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K, Shinozaki Y, Ohsawa K, Tsuda M, Joshi BV, Jacobson KA, Kohsaka S, Inoue K (2007) UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature 446(7139):1091–1095. https://doi.org/10.1038/nature05704
41. Frade JM, Barde YA (1998) Microglia-derived nerve growth factor causes cell death in the developing retina. Neuron 20(1):35–41. https://doi.org/10.1016/s0896-6273(00)80432-8
42. Murase S, Hayashi Y (1998) Expression pattern and neurotrophic role of the c-fms proto-oncogene M-CSF receptor in rodent Purkinje cells. J Neurosci 18(24):10481–10492
43. Del Rio P, Irmler M, Arango-Gonzalez B, Favor J, Bobe C, Bartsch U, Vecino E, Beckers J, Hauck SM, Ueffing M (2011) GDNF-induced osteopontin from Muller glial cells promotes photoreceptor survival in the Pde6brd1 mouse model of retinal degeneration. Glia 59(5):821–832. https://doi.org/10.1002/glia.21155
44. Nakajima K, Kohsaka S (2004) Microglia: neuroprotective and neurotrophic cells in the central nervous system. Curr Drug

Targets Cardiovasc Haematol Disord 4(1):65–84. https://doi.org/10.2174/1568006043481284
45. Goda Y, Davis GW (2003) Mechanisms of synapse assembly and disassembly. Neuron 40(2):243–264. https://doi.org/10.1016/s0896-6273(03)00608-1
46. Riccomagno MM, Kolodkin AL (2015) Sculpting neural circuits by axon and dendrite pruning. Annu Rev Cell Dev Biol 31:779–805. https://doi.org/10.1146/annurev-cellbio-100913-013038
47. Luo L, O'Leary DD (2005) Axon retraction and degeneration in development and disease. Annu Rev Neurosci 28:127–156. https://doi.org/10.1146/annurev.neuro.28.061604.135632
48. Milligan CE, Cunningham TJ, Levitt P (1991) Differential immunochemical markers reveal the normal distribution of brain macrophages and microglia in the developing rat brain. J Comp Neurol 314(1):125–135. https://doi.org/10.1002/cne.903140112
49. Hristova M, Cuthill D, Zbarsky V, Acosta-Saltos A, Wallace A, Blight K, Buckley SM, Peebles D, Heuer H, Waddington SN, Raivich G (2010) Activation and deactivation of periventricular white matter phagocytes during postnatal mouse development. Glia 58(1):11–28. https://doi.org/10.1002/glia.20896
50. Monier A, Evrard P, Gressens P, Verney C (2006) Distribution and differentiation of microglia in the human encephalon during the first two trimesters of gestation. J Comp Neurol 499(4):565–582. https://doi.org/10.1002/cne.21123
51. Verney C, Monier A, Fallet-Bianco C, Gressens P (2010) Early microglial colonization of the human forebrain and possible involvement in periventricular white-matter injury of preterm infants. J Anat 217(4):436–448. https://doi.org/10.1111/j.1469-7580.2010.01245.x
52. Squarzoni P, Oller G, Hoeffel G, Pont-Lezica L, Rostaing P, Low D, Bessis A, Ginhoux F, Garel S (2014) Microglia modulate wiring of the embryonic forebrain. Cell Rep 8(5):1271–1279. https://doi.org/10.1016/j.celrep.2014.07.042
53. Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, Yamashita T (2013) Layer V cortical neurons require microglial support for survival during postnatal development. Nat Neurosci 16(5):543–551. https://doi.org/10.1038/nn.3358
54. Pont-Lezica L, Beumer W, Colasse S, Drexhage H, Versnel M, Bessis A (2014) Microglia shape corpus callosum axon tract fasciculation: functional impact of prenatal inflammation. Eur J Neurosci 39(10):1551–1557. https://doi.org/10.1111/ejn.12508
55. Imamoto K, Leblond CP (1978) Radioautographic investigation of gliogenesis in the corpus callosum of young rats. II. Origin of microglial cells. J Comp Neurol 180(1):139–163. https://doi.org/10.1002/cne.901800109
56. Ling EA, Ng YK, Wu CH, Kaur C (2001) Microglia: its development and role as a neuropathology sensor. Prog Brain Res 132:61–79. https://doi.org/10.1016/S0079-6123(01)32066-6
57. Monier A, Adle-Biassette H, Delezoide AL, Evrard P, Gressens P, Verney C (2007) Entry and distribution of microglial cells in human embryonic and fetal cerebral cortex. J Neuropathol Exp Neurol 66(5):372–382. https://doi.org/10.1097/nen.0b013e3180517b46
58. Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314–1318. https://doi.org/10.1126/science.1110647
59. Chamak B, Morandi V, Mallat M (1994) Brain macrophages stimulate neurite growth and regeneration by secreting thrombospondin. J Neurosci Res 38(2):221–233. https://doi.org/10.1002/jnr.490380213
60. Morgan SC, Taylor DL, Pocock JM (2004) Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. J Neurochem 90(1):89–101. https://doi.org/10.1111/j.1471-4159.2004.02461.x
61. Ozdinler PH, Macklis JD (2006) IGF-I specifically enhances axon outgrowth of corticospinal motor neurons. Nat Neurosci 9(11):1371–1381. https://doi.org/10.1038/nn1789
62. Fujita Y, Nakanishi T, Ueno M, Itohara S, Yamashita T (2020) Netrin-G1 regulates microglial accumulation along axons and supports the survival of layer v neurons in the postnatal mouse brain. Cell Rep 31(4):107580. https://doi.org/10.1016/j.celrep.2020.107580
63. Arlotta P, Molyneaux BJ, Chen J, Inoue J, Kominami R, Macklis JD (2005) Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. Neuron 45(2):207–221. https://doi.org/10.1016/j.neuron.2004.12.036
64. Nakashiba T, Ikeda T, Nishimura S, Tashiro K, Honjo T, Culotti JG, Itohara S (2000) Netrin-G1: a novel glycosyl phosphatidylinositol-linked mammalian netrin that is functionally divergent from classical netrins. J Neurosci 20(17):6540–6550
65. Nakashiba T, Nishimura S, Ikeda T, Itohara S (2002) Complementary expression and neurite outgrowth activity of netrin-G subfamily members. Mech Dev 111(1–2):47–60. https://doi.org/10.1016/s0925-4773(01)00600-1
66. Yin Y, Miner JH, Sanes JR (2002) Laminets: laminin- and netrin-related genes expressed in distinct neuronal subsets. Mol Cell Neurosci 19(3):344–358. https://doi.org/10.1006/mcne.2001.1089
67. Arnoux I, Hoshiko M, Sanz Diez A, Audinat E (2014) Paradoxical effects of minocycline in the developing mouse somatosensory cortex. Glia 62(3):399–410. https://doi.org/10.1002/glia.22612
68. Hughes AN, Appel B (2020) Microglia phagocytose myelin sheaths to modify developmental myelination. Nat Neurosci 23(9):1055–1066. https://doi.org/10.1038/s41593-020-0654-2
69. Peferoen L, Kipp M, van der Valk P, van Noort JM, Amor S (2014) Oligodendrocyte-microglia cross-talk in the central nervous system. Immunology 141(3):302–313. https://doi.org/10.1111/imm.12163
70. Wang J, He X, Meng H, Li Y, Dmitriev P, Tian F, Page JC, Lu QR, He Z (2020) Robust myelination of regenerated axons induced by combined manipulations of GPR17 and microglia. Neuron. https://doi.org/10.1016/j.neuron.2020.09.016
71. Liu P, Du JL, He C (2013) Developmental pruning of early-stage myelin segments during CNS myelination in vivo. Cell Res 23(7):962–964. https://doi.org/10.1038/cr.2013.62
72. Kantor DB, Kolodkin AL (2003) Curbing the excesses of youth: molecular insights into axonal pruning. Neuron 38(6):849–852. https://doi.org/10.1016/s0896-6273(03)00364-7
73. Gu Z, Kalambogias J, Yoshioka S, Han W, Li Z, Kawasawa YI, Pochareddy S, Li Z, Liu F, Xu X, Wijeratne HRS, Ueno M, Blatz E, Salomone J, Kumanogoh A, Rasin MR, Gebelein B, Weirauch MT, Sestan N, Martin JH, Yoshida Y (2017) Control of species-dependent cortico-motoneuronal connections underlying manual dexterity. Science 357(6349):400–404. https://doi.org/10.1126/science.aan3721
74. Pauli A, Althoff F, Oliveira RA, Heidmann S, Schuldiner O, Lehner CF, Dickson BJ, Nasmyth K (2008) Cell-type-specific TEV protease cleavage reveals cohesin functions in *Drosophila* neurons. Dev Cell 14(2):239–251. https://doi.org/10.1016/j.devcel.2007.12.009
75. Watts RJ, Hoopfer ED, Luo L (2003) Axon pruning during *Drosophila* metamorphosis: evidence for local degeneration and requirement of the ubiquitin-proteasome system. Neuron 38(6):871–885. https://doi.org/10.1016/s0896-6273(03)00295-2
76. Lee T, Lee A, Luo L (1999) Development of the *Drosophila* mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Development 126(18):4065–4076
77. Awasaki T, Ito K (2004) Engulfing action of glial cells is required for programmed axon pruning during

Drosophila metamorphosis. Curr Biol 14(8):668–677. https://doi.org/10.1016/j.cub.2004.04.001
78. Watts RJ, Schuldiner O, Perrino J, Larsen C, Luo L (2004) Glia engulf degenerating axons during developmental axon pruning. Curr Biol 14(8):678–684. https://doi.org/10.1016/j.cub.2004.03.035
79. Awasaki T, Tatsumi R, Takahashi K, Arai K, Nakanishi Y, Ueda R, Ito K (2006) Essential role of the apoptotic cell engulfment genes draper and ced-6 in programmed axon pruning during *Drosophila* metamorphosis. Neuron 50(6):855–867. https://doi.org/10.1016/j.neuron.2006.04.027
80. Lee T, Marticke S, Sung C, Robinow S, Luo L (2000) Cell-autonomous requirement of the USP/EcR-B ecdysone receptor for mushroom body neuronal remodeling in *Drosophila*. Neuron 28(3):807–818. https://doi.org/10.1016/s0896-6273(00)00155-0
81. Reddien PW, Cameron S, Horvitz HR (2001) Phagocytosis promotes programmed cell death in *C. elegans*. Nature 412(6843):198–202. https://doi.org/10.1038/35084096
82. Tanaka T, Ueno M, Yamashita T (2009) Engulfment of axon debris by microglia requires p38 MAPK activity. J Biol Chem 284(32):21626–21636. https://doi.org/10.1074/jbc.M109.005603
83. Kole MH, Stuart GJ (2012) Signal processing in the axon initial segment. Neuron 73(2):235–247. https://doi.org/10.1016/j.neuron.2012.01.007
84. Baalman K, Marin MA, Ho TS, Godoy M, Cherian L, Robertson C, Rasband MN (2015) Axon initial segment-associated microglia. J Neurosci 35(5):2283–2292. https://doi.org/10.1523/JNEUROSCI.3751-14.2015
85. Tremblay ME, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A (2011) The role of microglia in the healthy brain. J Neurosci 31(45):16064–16069. https://doi.org/10.1523/JNEUROSCI.4158-11.2011
86. Yap EL, Greenberg ME (2018) Activity-regulated transcription: bridging the gap between neural activity and behavior. Neuron 100(2):330–348. https://doi.org/10.1016/j.neuron.2018.10.013
87. Tremblay ME, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8(11):e1000527. https://doi.org/10.1371/journal.pbio.1000527
88. Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J (2009) Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci 29(13):3974–3980. https://doi.org/10.1523/JNEUROSCI.4363-08.2009
89. Li Y, Du XF, Liu CS, Wen ZL, Du JL (2012) Reciprocal regulation between resting microglial dynamics and neuronal activity in vivo. Dev Cell 23(6):1189–1202. https://doi.org/10.1016/j.devcel.2012.10.027
90. Kato G, Inada H, Wake H, Akiyoshi R, Miyamoto A, Eto K, Ishikawa T, Moorhouse AJ, Strassman AM, Nabekura J (2016) Microglial contact prevents excess depolarization and rescues neurons from excitotoxicity. eNeuro. https://doi.org/10.1523/ENEURO.0004-16.2016
91. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74(4):691–705. https://doi.org/10.1016/j.neuron.2012.03.026
92. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D, Gross CT (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333(6048):1456–1458. https://doi.org/10.1126/science.1202529
93. Zhan Y, Paolicelli RC, Sforazzini F, Weinhard L, Bolasco G, Pagani F, Vyssotski AL, Bifone A, Gozzi A, Ragozzino D, Gross CT (2014) Deficient neuron–microglia signaling results in impaired functional brain connectivity and social behavior. Nat Neurosci 17(3):400–406. https://doi.org/10.1038/nn.3641
94. Hua JY, Smith SJ (2004) Neural activity and the dynamics of central nervous system development. Nat Neurosci 7(4):327–332. https://doi.org/10.1038/nn1218
95. Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274(5290):1133–1138. https://doi.org/10.1126/science.274.5290.1133
96. Sanes JR, Lichtman JW (1999) Development of the vertebrate neuromuscular junction. Annu Rev Neurosci 22:389–442. https://doi.org/10.1146/annurev.neuro.22.1.389
97. Feller MB (1999) Spontaneous correlated activity in developing neural circuits. Neuron 22(4):653–656. https://doi.org/10.1016/s0896-6273(00)80724-2
98. Huberman AD, Manu M, Koch SM, Susman MW, Lutz AB, Ullian EM, Baccus SA, Barres BA (2008) Architecture and activity-mediated refinement of axonal projections from a mosaic of genetically identified retinal ganglion cells. Neuron 59(3):425–438. https://doi.org/10.1016/j.neuron.2008.07.018
99. Shatz CJ, Kirkwood PA (1984) Prenatal development of functional connections in the cat's retinogeniculate pathway. J Neurosci 4(5):1378–1397
100. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, Sher A, Litke AM, Lambris JD, Smith SJ, John SW, Barres BA (2007) The classical complement cascade mediates CNS synapse elimination. Cell 131(6):1164–1178. https://doi.org/10.1016/j.cell.2007.10.036
101. Miyamoto A, Wake H, Ishikawa AW, Eto K, Shibata K, Murakoshi H, Koizumi S, Moorhouse AJ, Yoshimura Y, Nabekura J (2016) Microglia contact induces synapse formation in developing somatosensory cortex. Nat Commun 7:12540. https://doi.org/10.1038/ncomms12540
102. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR 3rd, Lafaille JJ, Hempstead BL, Littman DR, Gan WB (2013) Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 155(7):1596–1609. https://doi.org/10.1016/j.cell.2013.11.030
103. Ahuja CS, Wilson JR, Nori S, Kotter MRN, Druschel C, Curt A, Fehlings MG (2017) Traumatic spinal cord injury. Nat Rev Dis Primers 3:17018. https://doi.org/10.1038/nrdp.2017.18
104. Yiu G, He Z (2006) Glial inhibition of CNS axon regeneration. Nat Rev Neurosci 7(8):617–627. https://doi.org/10.1038/nrn1956
105. Filbin MT (2003) Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. Nat Rev Neurosci 4(9):703–713. https://doi.org/10.1038/nrn1195
106. Silver J, Schwab ME, Popovich PG (2014) Central nervous system regenerative failure: role of oligodendrocytes, astrocytes, and microglia. Cold Spring Harb Perspect Biol 7(3):a020602. https://doi.org/10.1101/cshperspect.a020602
107. Fujita Y, Yamashita T (2014) Axon growth inhibition by RhoA/ROCK in the central nervous system. Front Neurosci 8:338. https://doi.org/10.3389/fnins.2014.00338
108. Popovich PG, Guan Z, McGaughy V, Fisher L, Hickey WF, Basso DM (2002) The neuropathological and behavioral consequences of intraspinal microglial/macrophage activation. J Neuropathol Exp Neurol 61(7):623–633. https://doi.org/10.1093/jnen/61.7.623
109. Schwartz M (2003) Macrophages and microglia in central nervous system injury: are they helpful or harmful? J Cereb Blood Flow Metab 23(4):385–394. https://doi.org/10.1097/01.WCB.0000061881.75234.5E

110. Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8(1):57–69. https://doi.org/10.1038/nrn2038
111. Zecca L, Zucca FA, Albertini A, Rizzio E, Fariello RG (2006) A proposed dual role of neuromelanin in the pathogenesis of Parkinson's disease. Neurology 67(7 Suppl 2):S8-11. https://doi.org/10.1212/wnl.67.7_suppl_2.s8
112. McGeer PL, Rogers J, McGeer EG (2006) Inflammation, anti-inflammatory agents and Alzheimer disease: the last 12 years. J Alzheimers Dis 9(3 Suppl):271–276. https://doi.org/10.3233/jad-2006-9s330
113. Kim YS, Joh TH (2006) Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. Exp Mol Med 38(4):333–347. https://doi.org/10.1038/emm.2006.40
114. Hanisch UK (2002) Microglia as a source and target of cytokines. Glia 40(2):140–155. https://doi.org/10.1002/glia.10161
115. Hawthorne AL, Popovich PG (2011) Emerging concepts in myeloid cell biology after spinal cord injury. Neurotherapeutics 8(2):252–261. https://doi.org/10.1007/s13311-011-0032-6
116. Taylor DL, Jones F, Kubota ES, Pocock JM (2005) Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha-induced neurotoxicity in concert with microglial-derived Fas ligand. J Neurosci 25(11):2952–2964. https://doi.org/10.1523/JNEUROSCI.4456-04.2005
117. Kim SU, de Vellis J (2005) Microglia in health and disease. J Neurosci Res 81(3):302–313. https://doi.org/10.1002/jnr.20562
118. Fujita Y, Yamashita T (2014) Chapter 13. Microglia. Neuroprotection and regeneration for retinal diseases. Springer, Berlin
119. Paolicelli RC, Jawaid A, Henstridge CM, Valeri A, Merlini M, Robinson JL, Lee EB, Rose J, Appel S, Lee VM, Trojanowski JQ, Spires-Jones T, Schulz PE, Rajendran L (2017) TDP-43 depletion in microglia promotes amyloid clearance but also induces synapse loss. Neuron 95(2):297-308 e296. https://doi.org/10.1016/j.neuron.2017.05.037
120. Morioka T, Kalehua AN, Streit WJ (1993) Characterization of microglial reaction after middle cerebral artery occlusion in rat brain. J Comp Neurol 327(1):123–132. https://doi.org/10.1002/cne.903270110
121. Kato H, Kogure K, Liu XH, Araki T, Itoyama Y (1996) Progressive expression of immunomolecules on activated microglia and invading leukocytes following focal cerebral ischemia in the rat. Brain Res 734(1–2):203–212
122. Schilling M, Besselmann M, Muller M, Strecker JK, Ringelstein EB, Kiefer R (2005) Predominant phagocytic activity of resident microglia over hematogenous macrophages following transient focal cerebral ischemia: an investigation using green fluorescent protein transgenic bone marrow chimeric mice. Exp Neurol 196(2):290–297. https://doi.org/10.1016/j.expneurol.2005.08.004
123. Boekhoff TM, Ensinger EM, Carlson R, Bock P, Baumgartner W, Rohn K, Tipold A, Stein VM (2012) Microglial contribution to secondary injury evaluated in a large animal model of human spinal cord trauma. J Neurotrauma 29(5):1000–1011. https://doi.org/10.1089/neu.2011.1821
124. Jao LE, Wente SR, Chen W (2013) Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. Proc Natl Acad Sci USA 110(34):13904–13909. https://doi.org/10.1073/pnas.1308335110
125. Wahl AS, Omlor W, Rubio JC, Chen JL, Zheng H, Schroter A, Gullo M, Weinmann O, Kobayashi K, Helmchen F, Ommer B, Schwab ME (2014) Neuronal repair. Asynchronous therapy restores motor control by rewiring of the rat corticospinal tract after stroke. Science 344(6189):1250–1255. https://doi.org/10.1126/science.1253050
126. Lang C, Guo X, Kerschensteiner M, Bareyre FM (2012) Single collateral reconstructions reveal distinct phases of corticospinal remodeling after spinal cord injury. PLoS ONE 7(1):e30461. https://doi.org/10.1371/journal.pone.0030461
127. Bareyre FM, Kerschensteiner M, Raineteau O, Mettenleiter TC, Weinmann O, Schwab ME (2004) The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. Nat Neurosci 7(3):269–277. https://doi.org/10.1038/nn1195
128. Li Y, He X, Kawaguchi R, Zhang Y, Wang Q, Monavarfeshani A, Yang Z, Chen B, Shi Z, Meng H, Zhou S, Zhu J, Jacobi A, Swarup V, Popovich PG, Geschwind DH, He Z (2020) Microglia-organized scar-free spinal cord repair in neonatal mice. Nature. https://doi.org/10.1038/s41586-020-2795-6
129. Tran AP, Warren PM, Silver J (2018) The biology of regeneration failure and success after spinal cord injury. Physiol Rev 98(2):881–917. https://doi.org/10.1152/physrev.00017.2017
130. Prewitt CM, Niesman IR, Kane CJ, Houle JD (1997) Activated macrophage/microglial cells can promote the regeneration of sensory axons into the injured spinal cord. Exp Neurol 148(2):433–443. https://doi.org/10.1006/exnr.1997.6694
131. Batchelor PE, Porritt MJ, Martinello P, Parish CL, Liberatore GT, Donnan GA, Howells DW (2002) Macrophages and microglia produce local trophic gradients that stimulate axonal sprouting toward but not beyond the wound edge. Mol Cell Neurosci 21(3):436–453. https://doi.org/10.1006/mcne.2002.1185
132. Bellver-Landete V, Bretheau F, Mailhot B, Vallieres N, Lessard M, Janelle ME, Vernoux N, Tremblay ME, Fuehrmann T, Shoichet MS, Lacroix S (2019) Microglia are an essential component of the neuroprotective scar that forms after spinal cord injury. Nat Commun 10(1):518. https://doi.org/10.1038/s41467-019-08446-0
133. Fu H, Zhao Y, Hu D, Wang S, Yu T, Zhang L (2020) Depletion of microglia exacerbates injury and impairs function recovery after spinal cord injury in mice. Cell Death Dis 11(7):528. https://doi.org/10.1038/s41419-020-2733-4
134. Matsuda T, Irie T, Katsurabayashi S, Hayashi Y, Nagai T, Hamazaki N, Adefuin AMD, Miura F, Ito T, Kimura H, Shirahige K, Takeda T, Iwasaki K, Imamura T, Nakashima K (2019) Pioneer factor NeuroD1 rearranges transcriptional and epigenetic profiles to execute microglia-neuron conversion. Neuron 101(3):472-485 e477. https://doi.org/10.1016/j.neuron.2018.12.010
135. Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, Walker AJ, Gergits F, Segel M, Nemesh J, Marsh SE, Saunders A, Macosko E, Ginhoux F, Chen J, Franklin RJM, Piao X, McCarroll SA, Stevens B (2019) Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. Immunity 50(1):253-271 e256. https://doi.org/10.1016/j.immuni.2018.11.004
136. Masuda T, Sankowski R, Staszewski O, Bottcher C, Amann L, Sagar SC, Nessler S, Kunz P, van Loo G, Coenen VA, Reinacher PC, Michel A, Sure U, Gold R, Grun D, Priller J, Stadelmann C, Prinz M (2019) Author correction: spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. Nature 568(7751):E4. https://doi.org/10.1038/s41586-019-1045-2
137. Masuda T, Sankowski R, Staszewski O, Bottcher C, Amann L, Sagar SC, Nessler S, Kunz P, van Loo G, Coenen VA, Reinacher PC, Michel A, Sure U, Gold R, Grun D, Priller J, Stadelmann C, Prinz M (2019) Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. Nature 566(7744):388–392. https://doi.org/10.1038/s41586-019-0924-x
138. Sankowski R, Bottcher C, Masuda T, Geirsdottir L, Sagar SE, Seredenina T, Muhs A, Scheiwe C, Shah MJ, Heiland DH, Schnell O, Grun D, Priller J, Prinz M (2019) Mapping microglia states in the human brain through the integration of

high-dimensional techniques. Nat Neurosci 22(12):2098–2110. https://doi.org/10.1038/s41593-019-0532-y

139. Li Q, Cheng Z, Zhou L, Darmanis S, Neff NF, Okamoto J, Gulati G, Bennett ML, Sun LO, Clarke LE, Marschallinger J, Yu G, Quake SR, Wyss-Coray T, Barres BA (2019) Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing. Neuron 101(2):207–22310. https://doi.org/10.1016/j.neuron.2018.12.006