Review

Microglia: actively surveying and shaping neuronal circuit structure and function

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The traditional role of microglia has been in brain infection and disease, phagocytosing debris and secreting factors to modify disease progression. Recent evidence extends their role to healthy brain homeostasis, including the regulation of cell death, synapse elimination, neurogenesis, and neuronal surveillance. These actions contribute to the maturation and plasticity of neural circuits that ultimately shape behavior. Here we review microglial contributions to the development, plasticity, and maintenance of neural circuits with a focus on interactions with synapses. We introduce this topic by reviewing recent studies on the migration and proliferation of microglia within the brain, and conclude with the proposal that microglia dysfunction may adversely affect brain function, and thereby contribute to the development of psychiatric and neurological disorders.

Introduction

Microglia were first characterized by Pio del Rio-Hortega in the 1920s and 1930s, who described their distinct morphological phenotypes [1]. Many subsequent studies have focused on microglia and their roles in different brain pathologies [2,3], where a marked transformation of microglia takes place from a ramified to an amoeboid morphology, associated with the secretion of neuroactive compounds, the expression of various cell-surface receptors, proliferation, and phagocytosis. This has resulted in the traditional view that microglial function is largely associated with disease, but with the implication that microglial involvement in any pathology is secondary to disease formation and progression. Indeed, the observed phenotypic changes have led to a concept of dormant ramified or 'resting' microglia in the healthy adult brain, and that only amoeboid or 'activated' microglia influence brain function and pathology. However, this is clearly not entirely true, and recent studies indicate that both 'resting' and 'activated' microglia (as defined by this morphological phenotype) have physiological functions even in the absence of pathologies. Consequently the concept of resting and activated is misleading because multiple phenotypic stages of microglia can influence neuronal structure and function. In this review we highlight recent findings that have advanced our understanding of microglia in normal central nervous system (CNS) homeostasis, and suggest that microglia function to maintain neural circuits. We further speculate that dysfunction of this normal homeostatic role may contribute to disease.

Immigrants to the CNS take up long-term residence

The embryonic origins of microglia have been hotly debated over the past several decades [4]. The genetic and immunohistochemical fingerprint of microglia strongly supports the consensus that they are not derived from the same embryonic lineage (neuroectoderm) as neurons and astrocytes, but instead share the same mesodermal origin as macrophages and other hematopoietic cells [5–8]. For example, mice deficient in PU.1, a transcription factor which controls the differentiation of myeloid cells into macrophages, lack microglia [9]. Microglia migrate from this lineage into the CNS in early embryogenesis: macrophage progenitors are found in the neuroepithelium of mice at embryonic (E) days E8.5–10 [10,11], with substantial numbers being detected in the fourth ventricle at E10.5 [11]. In rodents, peripheral macrophages and other myeloid cells are derived from both hematopoietic stem cells (HSCs) and from a second population of yolk-sac progenitors which differentiate slightly earlier. Fate-mapping using fluorescent traces expressed under the control of an age-dependent promoter (i.e., *Runx1*) indicates that the adult microglia population are all derived from embryonic progenitors present at E7.5 in the mouse [12], suggesting that this earlier differentiating yolk-sac population is the major source of the resident adult microglia population. HSC-derived myeloid cells are dependent on a specific transcription factor, MyB, for their maintenance and

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renewal in the adult, whereas yolk sac-derived monocytes persist in the absence of MyB [13,14]. Microglia in the adult brain parenchyma were unaffected by loss of MyB, confirming that they derive from this earlier yolk-sac population [13,14]. Following migration, microglia populate the CNS with extensive proliferation [11,15]. During postnatal (P) days P0–11 a 20-fold increase in the number of microglia has been reported [16]. This migration and subsequent mitotic phase is of prime importance for the subsequent functions of microglia because the adult population in healthy brain is maintained by proliferation instead of by infiltration of circulating myeloid progenitors [12,14,17]. Runx1 is an important transcription factor that regulates the balance between amoeboid microglia, which characterize the neonatal proliferative phase (and the injured brain), and the ramified phenotype, which dominates the (healthy) adult brain [18]. Macrophage colony-stimulating factors (CSFs) have also been implicated in the control of microglial proliferation [19–21]. Determining the mechanisms that control this developmental proliferation and differentiation will be important for understanding microglial responses to brain disturbances.

Source of microglial expansion in disease

An increase in the number of microglia occurs in response to brain injuries. Such reactive microgliosis is a feature of both acute injury and chronic or recurring neuronal diseases, including infections, facial nerve axotomy, Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and stroke [1,19,21]. The increased microglial population stems from a variable mix of infiltration of circulating monocytes, proliferation of the resident population, and/or from migration from uninjured regions. The extent to which these sources contribute varies with different injuries – facial nerve axotomy largely involves proliferation of resident microglia, whereas the entorhinal cortex lesion model largely involves migration [22] and infiltration of circulating monocyte [23]. Infiltration of circulating monocytes is particularly evident when the vasculature is disrupted, such as in trauma and stroke, although it is not exclusive to diseases that disrupt the blood–brain barrier (BBB) [4,19]. Circulating monocytes can enter the CNS in experimental autoimmune encephalitis (EAE), although they did not persist and differentiate into unchallenged, ramified microglia [24]. In diseases such as AD, PD, and ALS the BBB is intact, which presents a very different scenario compared to EAE or trauma. Several studies addressing the source of microgliosis have used bone-marrow irradiation and donor transplantation, a procedure which itself can disrupt the BBB to facilitate infiltration of circulating monocytes (e.g., [12]). Indeed, circulating monocytes were found to make only a very small or negligible contribution to the local expansion of microglia in experimental denervation or in neurodegenerative disease [17]. Interestingly, the incidence of microgliomas is a very rare cause of CNS tumors, as compared to astrocytomas or oligodendrocytomas, suggesting a very tight regulation of microglial cell cycle [25]. Determining the extent of peripheral infiltration in specific conditions is an important consideration for the treatment of neurodegenerative and autoimmune diseases, and may also be a means to access the focus of CNS injury for gene therapy or drug delivery.

Microglial regulation of neuronal numbers in development and in the adult CNS

Developmental programmed cell death

Following their establishment in the CNS, microglia regulate neuronal circuit development. Programmed cell death (PCD) is an integral part of the refinements that take place during CNS development [26]. Amoeboid microglia have long been known to phagocytose the apoptotic neurons associated with PCD [10,27,28], but the role of microglia extends beyond simply cleaning up the debris. In developing vertebrate spinal cord, cerebellum, and hippocampus, microglia play a more active role in that they promote PCD [29–31]. Several signaling interactions are involved, including microglia responding to neuronal 'eat-me' signals, microglia priming neurons for PCD, and microglia directly triggering PCD by the release of neurotoxic substances [30]. For example, the majority of cerebellar Purkinje cells undergoing developmental apoptosis are engulfed by amoeboid microglia that release superoxide ions to trigger this PCD [31]. Abnormalities of cerebellar development, including alterations in neuronal numbers and neural circuit formation, result in deficiency of sensory motor coordination and cerebellar ataxia [32]. Given their role in cerebellar PCD, it would be interesting to investigate if abnormalities in microglia function could contribute to these (and other) developmental disorders. For example, a recent study reported elevated interleukin-6 (IL-6) levels in cerebellar tissues from autistic children. When IL-6 was overexpressed in cultured cerebellar neurons and microglia, there was altered cell adhesion, migration, and synaptogenesis [33].

Adult neurogenesis

Only a small subset of neural progenitor cells arising from the subgranular zone of the hippocampal dentate gyrus survive to become integrated into neuronal circuits [34]. The majority die by apoptosis in the first few days, being engulfed and phagocytosed by microglia [35]. Interestingly, this phagocytosis is by ramified or unchallenged 'resting' microglia, as opposed to the amoeboid microglia that typically mediate phagocytosis. Furthermore, phagocytosis by these ramified microglia involved the formation of a phagocytic pouch in distal extensions [35], as opposed to engulfment by the amoeboid cell soma. This again emphasizes the traditional labeling of ramified microglia as 'resting' is deceptive.

Microglia also release soluble substances, such as neurotrophic factors, to affect the extent of proliferation of neural progenitor cells [36]. Given that the extent of neurogenesis and/or progenitor proliferation is increased in epilepsy [37], neurodegenerative disease [38], and stroke [39], and decreased in AD [40,41] and drug addiction [42], targeting microglia regulation of these processes may become a strategy to modulate the progression of these diseases. Hippocampal neurogenesis is also part of normal brain homeostasis, being affected by environmental enrichment, learning, and exercise [43]. The stimulation of neurogenesis and microglia proliferation by environmental

enrichment, and the subsequent increase in spatial learning abilities, are impaired in immune-deficient mice [44]. This study proposed that T cells infiltrated the neurogenic niches of the subventricular and subgranular zones to activate microglia and enhance neurogenesis, suggesting more broadly that immune status may affect learning and cognition. Genetic knockout of the microglial chemokine receptor, Cx3CR1, resulted in decreased neurogenesis and impairments in spatial learning and other behavioral and learning tasks [45]. More directly, microglia derived from exercised mice have been shown to enhance the number of neural progenitor cells in cultured hippocampal neurons derived from sedentary mice [46]. These results implicate microglia in the maintenance and plasticity of neuronal circuits that are important for information processing and for learning and memory. Interestingly, depletion of microglia from cultures derived from aged mice conversely reduced the age-related decline in neurogenesis [46], indicating that microglia can differentially regulate neurogenesis.

In conclusion, ramified or unchallenged microglia are implicated in adult neurogenesis and in the integration of these new neurons into neuronal circuits. This neuronal plasticity is important for learning, memory, and cognition.

Interactions between microglia and synapses in neural circuit formation and maintenance

The discussion above has focused on the role of microglia in regulating neuronal numbers during development and in the adult CNS. Neurons also need to be correctly wired up in neuronal circuits to mediate brain functions. This occurs during development in an activity-dependent fashion as excessive synapses are eliminated or pruned, while key functional synapses are strengthened [47,48]. Incorrect wiring of synapses and altered synapse morphology can be associated with developmental disorders such as the autism spectrum disorders (ASDs) and with psychiatric diseases such as schizophrenia and depression [49–52].

Microglia and synaptic stripping

The facial nerve axotomy model has become a useful model in which to investigate the neuronal and synaptic plasticity that occurs in response to injury [53]. Axotomy of the facial nerve results in a range of cellular changes that ultimately result in apoptosis of a subset of the relevant motor neurons. Loss of afferent synaptic inputs is one of the earliest changes, and this has been proposed to prevent hyperactivation of motor neurons and facilitate their survival [53,54]. Resident microglia markedly proliferate in the vicinity of the motor nuclei in response to the axotomy [54,55], and their processes become interspersed between the pre- and postsynaptic elements, suggesting they are literally 'stripped' off these synapses [56] (Figure 1a). Activation of microglia by cerebral inflammation also results in substantial loss of synapses on adjacent neuronal somas, suggesting a role of microglia in initiating synaptic stripping [57] (Figure 1b). Astrocytes, however, also show biochemical and morphological changes in response to axotomy, and may be more important in the protection of motor neurons from apoptosis [58]. The relative contributions of these glial cells, and any interactions between them in injury-induced loss of synapses, needs further exploration, with species differences being an important point to recognize [54,55]. Recently, the acute effects of microglia activation by the proinflammatory molecule lipopolysaccharide (LPS) on synaptic transmission in brain slices was linked to astrocytes [59]. Bath application of LPS increased spontaneous excitatory postsynaptic currents and decreased the threshold for seizure-like bursting. A variety of pharmacological and genetic manipulations have demonstrated that microglial activation resulted in ATP release and the activation of purinergic receptors (P2Y1) on astrocytes, and this resulted in the subsequent release of glutamate and activation of presynaptic metabotropic glutamate receptors (mGluRs) [59]. There are a number of other examples of important and close interplay between different glial cells in neuronal circuits [60,61].

Direct interactions between microglia and neurons contribute to synaptic homeostasis and developmental plasticity

Interactions between microglia and neurons at synaptic sites have been directly visualized *in vivo* using two-photon microscopy [62–65]. The initial study was performed in young transgenic mice (aged 6–10 weeks) in which microglia were labeled by a fluorescent tag driven by the ionized Ca^{2+}-binding adapter molecule 1 (*Iba1*) promoter [66], coupled with sparsely tagged layer 5 cortical neurons (M-line mice) [67]. Contacts between microglial processes and presynaptic and postsynaptic neuronal elements were directly imaged and semi-quantified. Microglia made frequent but brief contacts with synapses in control (i.e., anaesthetized) conditions (Figure 1c). When brains were made ischemic during the recording period by photothrombosis, the duration of these contacts increased markedly (from ~5 to ~60 min) in the penumbra of the ischemic cortex [65]. Ischemia results in a higher turnover rate of presynaptic boutons and postsynaptic spines, and some synapses that had been contacted by microglia were subsequently lost [65]. It was suggested that microglia detected the condition of synapses, and could identify synapses to be eliminated during post-ischemia circuit remodeling.

Two subsequent studies confirmed these interactions between microglia and synapses using high-resolution microscopy. 3D reconstruction of serial-section electron microscopy revealed extensive contacts in the visual cortex of juvenile (4 weeks of age) mice between microglia and axon terminals, spines, perisynaptic astrocytes, and with the synaptic cleft. Almost every microglial cell made at least one contact, and often multiple contacts, with these elements [64] (Figure 1d). Time-lapse two-photon imaging revealed that contacted spines were more likely to be subsequently eliminated, further supporting a role of microglia in structural plasticity of synapses [64]. Stimulated-emission depletion (STED) microscopy and immunogold electron-microscopy data directly demonstrated that microglia engulfed synapses in the postnatal (P15) mouse hippocampus [68]. Both presynaptic and postsynaptic immunogold reactivity [measured by synaptosomal-associated protein 25 (SNAP25) and postsynaptic density protein 95 (PSD95)-immunoreactivity, respectively] were

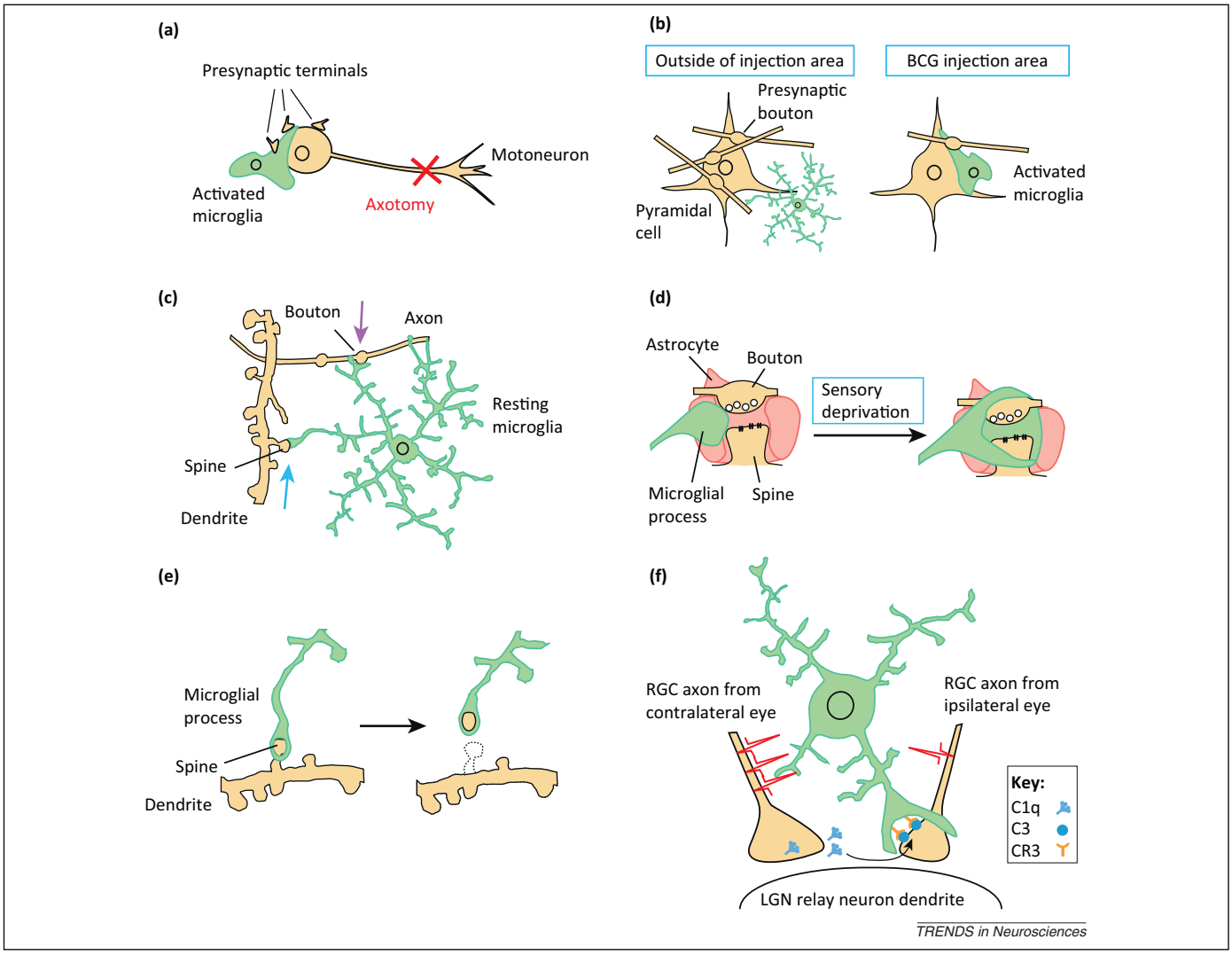

Figure 1. Schematic summary of microglia and synapse interactions. **(a)** Axotomy of the facial nerve induces 'synaptic stripping' of presynaptic excitatory afferent terminals projecting to the facial motor neurons, resulting in reduced neuronal hyperactivity. Microglia are strongly associated with this synaptic stripping, and this was the first description of specific microglia–synapse interactions [56]. The schematic image illustrates microglial processes invading into the synaptic cleft and inserting between the synapse and soma. **(b)** Microglia responding to cerebral inflammation [in response to heat-killed Bacillus Calmette–Guerin (BCG) treatment] reduced the number of axo-somatic synapses on adjacent neurons, providing wider evidence that microglia can influence synaptic number [57]. The schematic illustrates a neuronal cell body contacted by microglia with a much lower density of synapses as compared to neurons further away from the microglial contact region. This suggests that the responding microglia have removed the synapses. **(c)** Direct contacts between resting microglia and presynaptic terminal boutons and postsynaptic spines observed *in vivo* by the use of adapted two-photon microscopy and double-labeled transgenic mice. The schematic image shows microglia contacting both presynaptic terminals (purple arrow) and a postsynaptic spine (blue arrow). Note bulb formation at the tip of the microglial process that forms before contact with synaptic structures [65]. **(d)** The contacts between microglia and synapses are affected by physiological sensory inputs [64]. A schematic image demonstrating a microglia in direct contact with synapses, and also with an astrocyte. Sensory deprivation results in increased microglia–synapse contacts. **(e)** Microglia have been directly demonstrated to phagocytose synapses in the developing mouse hippocampus, engulfing both pre- and postsynaptic elements [68]. The schematic image shows part of the dendritic spine being engulfed by microglia. **(f)** The pruning of retinal ganglion cell (RGC) synapses is activity-dependent, with RGC inputs from the eye made quiescent by tetrodotoxin injection (i.e., weak synapses) undergoing an increased microglial engulfment, whereas inputs from afferents that were stimulated to increase their neuronal activity by forskolin injections (i.e., strong synapses) were maintained and had a greater dominance within the target lateral geniculate nucleus (LGN). This pruning of synapses in the LGN was mediated by the complement protein C3 and signaling via its receptor, CR3 [69].

detected inside microglia [68] (Figure 1e). In the hippocampus of a chemokine receptor (Cx3CR1) knockout mouse there was a transient decrease in microglia density and a corresponding transient increase in spine density, indicating that engulfment of synapses by microglia was a critical component of the developmental pruning of synapses that is required for circuit maturation [68]. Consistently, excitatory synaptic transmission to CA1 neurons showed a more immature phenotype at this time-period in the knockout mice [68]. Microglia also engulf retinal ganglion cell synapses in the lateral geniculate nucleus during developmental pruning in the mouse visual system [69] (Figure 1f) (discussed further below). Together, these findings suggest that microglia–synapse interactions significantly contribute to synaptic pruning during circuit maturation, in synapse surveillance in the healthy brain, and in the rewiring of neuronal circuits following injury.

Activity-dependent interactions between microglia and neural circuits

The close interactions between microglia and synapses imply that signaling may take place between these elements. Modulating electrical activity has given inconsistent results on microglial motility and synapse

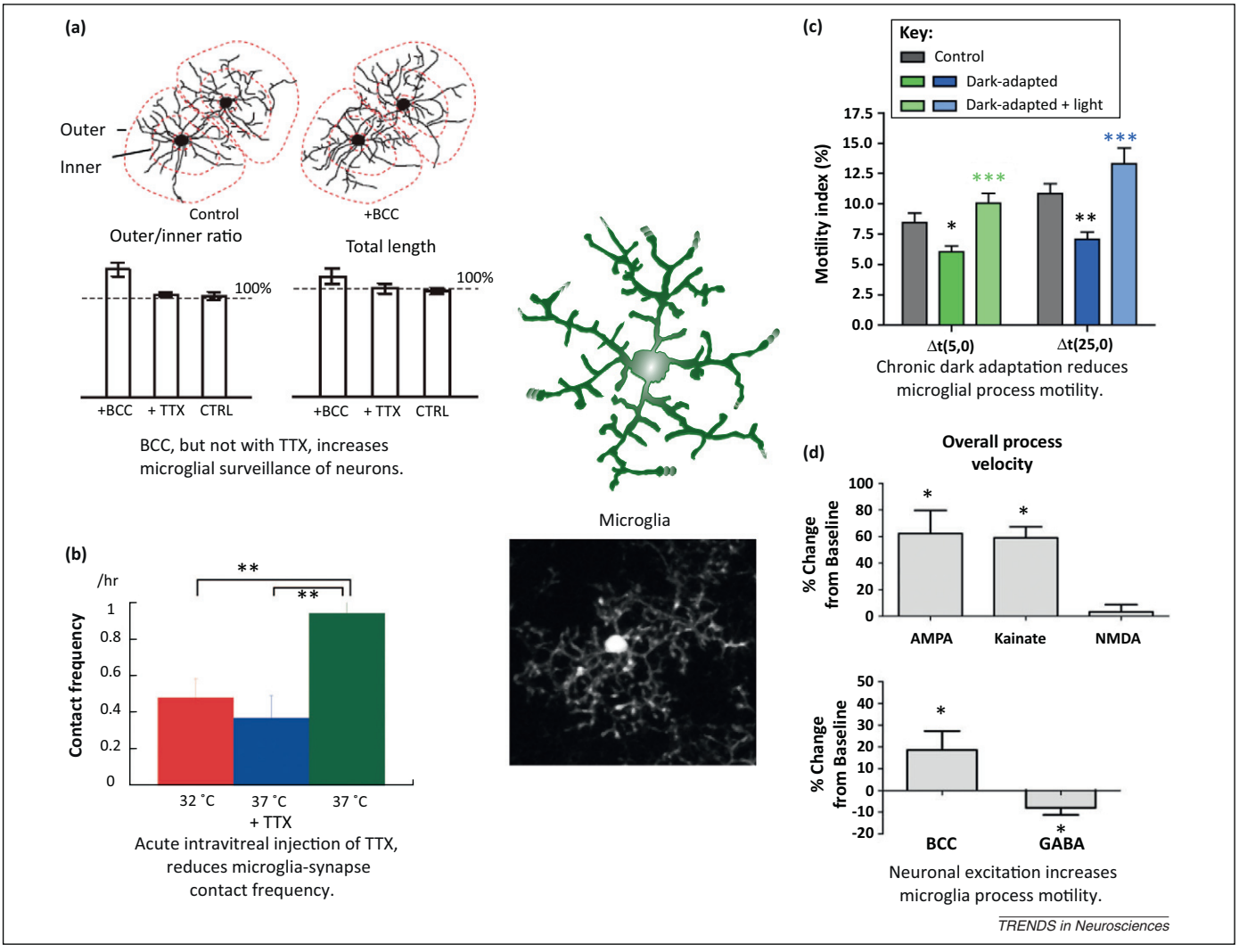

Figure 2. Microglia–synapse interactions and neuronal activity. A summary of the effects of acute and chronic pharmacological, physiological and pathological modulation of neuronal activity on microglia–synapse contacts. (Middle panel) The schematic and the photomicrograph show a ramified quiescent microglia from an *in vivo* two-photon image [65]. **(a)** Application of the $GABA_A$ receptor antagonist, bicucculline (BCC), to the mature mouse brain surface *in vivo* results in increased neuronal activity (not shown) and an increase in the extent of microglial surveillance of neurons [70]. Microglial process surveillance was measured as microglial process fluorescence in an outer ring relative to an inner ring, as shown by the dotted spheres and quantified in the bar graphs. However, tetrodotoxin (TTX), which inhibited neuronal activity, had no effect on surveillance. Microglial process motility was unaffected by both treatments. Surface application of saline (CTRL) had no effect on either parameter. **(b)** Manipulating neuronal activity via changes in body temperature or via pharmacological sensory deprivation had more consistent effects on microglia–synapse contact frequency. Sensory deprivation by intravitreal TTX injection, and reduction of body temperature to reduce neural activity, were both followed by a decrease in the frequency of microglia and synapse contacts [65]. **(c)** Chronic dark rearing, which is also thought to reduce the extent of neuronal activity, reduced microglial motility and this was reversed (and over-compensated for) by light activation. Dark-adapted (dark rearing: middle bar) mice showed reduced microglia process motility as compared with control (left bar), and this could be recovered by light activation (right bar). The two sets of bar graphs show the same data but at different timepoints, 5 and 25 min apart [64]. **(d)** (Top) Microglial motility is increased by application of the glutamate receptor agonists AMPA and kainate (but not NMDA), due to a signal transmitted from neurons via astrocytes. Neuronally released glutamate induces ATP release from astrocytes, which then directly acts on microglia to increase their motility. (Bottom) $GABA_A$ receptor activation (via the application of GABA) decreases general neural activity, decreases glutamate release from neurons, followed by a reduction of ATP release from astrocytes and reduced microglial motility [72]. By contrast, $GABA_A$ receptor inhibition (via BCC treatment) increases motility. Reproduced, with permission, from [70] (a), [65] (b), [64] (c) [72] (d).

interactions. Surface application of tetrodotoxin (TTX) to the mouse cortex had no effect on microglial process motility, whereas the $GABA_A$ receptor blocker bicuculline increased motility [70] (Figure 2a). In hippocampal slices, microglial motility did not change after induction of long-term potentiation by high-frequency stimulation [71]. However, reductions of neuronal activity in the visual cortex by eye enucleation, or by intravitreal TTX injection, decreased the frequency of microglia–synapse contacts (from ~1/h to ~0.4/h), without marked changes in motility [65] (Figure 2b). Sensory deprivation by dark rearing for 10 days decreased microglia motility but did not affect contact frequency [64] (Figure 2c). In an *ex vivo* retinal explant model, application of GABA decreased microglia motility, whereas application of glutamate increased motility [72]. These were indirect effects through activity-induced ATP release via pannexin channels [72] (Figure 2d). The results suggest some communication between microglia and other synaptic elements (i.e., astrocytes and/or neurons), and it will be important to characterize such signaling processes under different conditions.

Immune/complement molecules and microglia–synapse interactions

Studies on the molecular mechanisms mediating these tight interactions between microglia and synapses have demonstrated some homologies with the peripheral immune system. Proteins of the major histocompatibility

complex class I (MHC-1) and complement cascade (C1q and C3) are expressed in various neurons in an activity-dependent fashion [73,74]. A classic model of developmental synaptic pruning is the segregation of projections from retinal ganglion cells (RGCs) of each eye into the appropriate regions in the lateral geniculate nucleus (LGN). The expression of MHC-1 molecules is reduced when neuronal activity in the visual pathway is blocked, a manipulation which also disrupts this synaptic pruning [73]. A more direct role for the MHC-1 pathway comes from knockout mice. Loss of β2m, a light-chain protein found in most MHC-1 complexes, or the transporter associated with the antigen processing 1 (TAP1), a protein involved with loading of peptides on to MHC-1 complexes, results in reduction of this pruning and segregation [75]. MHC-1 molecules and some of their receptors are upregulated after axotomy, but an increased loss of synapses is seen in β2m- or TAP-1-deficient mice following axotomy [76]. The excess loss of synapses in the absence of MHC-1 mostly involved inhibitory terminals, suggesting a specific role of MHC-1 signaling in preserving inhibitory inputs [77]. The precise mechanisms by which specific synapses are identified for elimination, and the contribution of microglia, requires further investigation.

Mice devoid of genes for the classic complement proteins C1q and C3 also show reduced developmental pruning, with LGN neurons remaining multi-innervated by RGC inputs [74]. By analogy with the innate immune system, where C1q protein initiates a cascade in which C3 tags synapses for phagocytosis, these complement molecules were proposed to target synapses for pruning by microglia [69]. Consistently, the receptor for C3 is transiently upregulated in microglia during this peak period of synaptic pruning. Furthermore, mice deficient in C3, or in the α subunit of the C3 receptor, had a significantly reduced capacity for microglia engulfment of RGC afferent synapses [69].

Microglia and psychiatric disorders

The neurotoxic or neuroprotective roles of microglia in neurodegenerative diseases have been well documented (e.g., [1,78]). Microglia also play an important role in shaping synapses and neuronal circuits during development and neurogenesis, and survey the resting adult brain, as described above (Figure 3). If these microglial functions are disrupted, one can predict that this will result in dysfunction of the brain and in the emergence of psychiatric and neurological disorders (Figure 3). Hence, we propose dual facets to microglial contributions to neuronal dysfunction: (i) alterations in microglia function in the uninjured brain can contribute to psychiatric and neurological diseases, and (ii) challenged microglia can affect the progression and/or outcome of an established neuronal disease. It remains speculative whether alterations in microglia cause brain disease, but support for this scenario has recently emerged from different hereditary disorders.

Rett syndrome is an ASD with a clearly defined genetic basis [79]. Mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene result in altered expression of MeCP2. In addition to being expressed in neurons, MeCP2 is also expressed in glial cells (including microglia) and at least some of the focus for the pathophysiology of Rett syndrome has consequently shifted to include a potential contribution of glia [80–83]. The symptoms of Rett disease in the

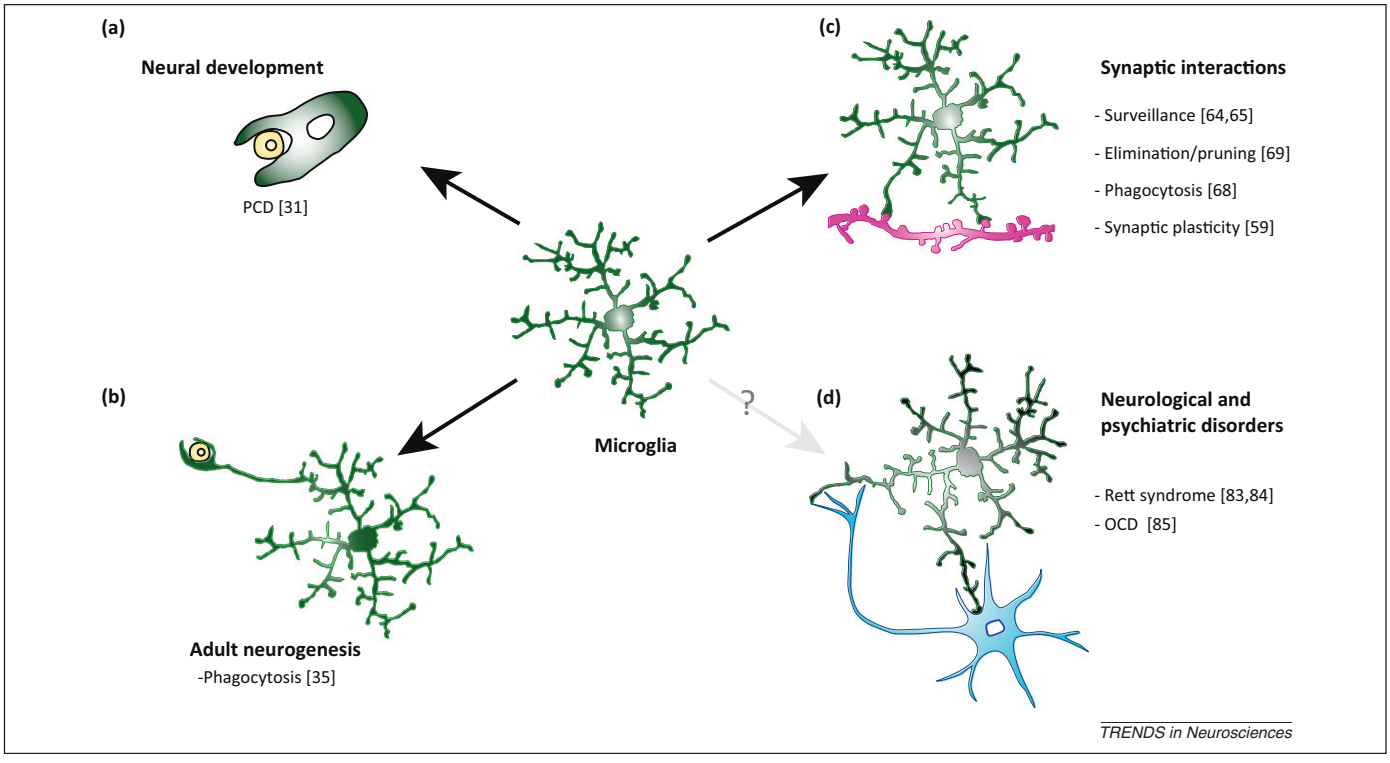

Figure 3. Physiological roles of microglia. Schematic diagrams of the multiple postulated physiological functions of microglia in forming and maintaining neuronal circuits. These physiological functions include **(a)** regulating programmed cell death (PCD) during development, **(b)** regulation of neurogenesis, including the phagocytosis of adult new-born cells, and **(c)** the surveillance, monitoring, and pruning of presynaptic terminals and dendritic spines to maintain homeostasis of synapses. **(d)** Microglia have been demonstrated to be associated with a number of neurological and psychiatric disorders, including Rett syndrome [83,84] and obsessive-compulsive disorder (OCD) [85]. It is tempting to speculate that microglia could be involved in the pathogenesis of neurological disorders if any of these important physiological functions are altered. Key: blue, neuron; green, microglia; grey, dysfunctional microglia; red, synapses on dendrites; yellow, neural lineage cells.

MeCP2 null mouse are ameliorated by irradiation and bone-marrow transplantation [84], a procedure known to replenish the microglia population. The disease was also ameliorated in a MeCP2^{lox}/Lysm^{cre} transgenic mouse, in which expression of MeCP2 was restored specifically in myeloid cells, supporting the locus of effect to microglia. It was concluded that disease progression was inhibited by restoring microglial phagocytic capacity to reduce the accumulation of debris [84]. However, microglia–synapse interactions may have also contributed to disease inhibition. The time-window in which microglia could reduce disease progression (i.e., P28 but not P40) [84] would be consistent with an effect on synapse maturation.

The pathogenesis of a mouse model of obsessive compulsive disorder (OCD) has also been linked to microglia. A loss of function mutation in the transcription factor gene, *Hoxb8*, results in obsessive grooming and hair pulling in mice, and this has been associated with loss of Hox8b in microglia [85]. The phenotype was rescued by irradiation and bone-marrow transplantation, and was replicated in a microglia-specific knockout of Hox8b [85]. Puzzlingly, the Hox8b-deficient microglia (which accounted for about 40% of the adult microglia population) were traced to a hematopoietic bone marrow-derived cell lineage which appeared later in development (i.e., from P2) [85] instead of to the yolk-sac progenitors that have been shown to constitute the adult resident microglia population (see above discussion). Hence, additional studies are needed to clarify these seemingly contradictory findings.

In another example, mutations affecting a CSF (i.e., CSF-1) have been linked to a late-onset neurodegenerative disease known as hereditary diffuse leukoencephalopathy with spheroids (HDLS) [86], which is characterized by symptoms that include depression, anxiety, aggressiveness, and dementia [87]. CSF-1 is an important regulator of microglial proliferation and differentiation, and it was proposed that the primary pathogenesis of the disorder was dysfunction of microglial reactivity. Mutations in CSF-1 or its signaling pathways have been also observed in some other neurological disorders, including frontal temporal lobe dementia and Nasu–Hokala disease [86,87]. The functional consequences of altered CSF-1 signaling in these disorders, and selective rescue by normal microglia, have yet to be investigated.

Taken together, the results related to these hereditary disorders suggest that microglial dysfunction may precipitate neuronal disease. Early-life activation of microglia by LPS, infection, or stress can result in cognitive disabilities that can persist through to adulthood [88]. Depression, ASDs, bipolar disorder, and schizophrenia are known to be associated with microglial activation [87,88], but whether this contributes to their pathogenesis is unknown. It has been suggested that altered reactivity of microglia may couple with other environmental and genetic factors to precipitate schizophrenia and depression [88]. We further speculate that altered homeostatic functions of resting microglia may also contribute to neurological and psychiatric disorders, either directly or via interactions with other environmental and/or genetic factors. In schizophrenia, functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have revealed both a wider range of spontaneous cortical activity and a greater variability in evoked cortical response patterns as compared to control subjects [89,90]. At the morphological level, schizophrenia is associated with a decreased number of synapses in affected regions [51,91]. Spine morphological changes have been also seen in schizophrenia [92,93] and in depression [94]. Decreases in the number of synaptic spines have also been recently reported in major depressive disorder [95]. It is interesting to speculate that microglial regulation of synapse number or morphology may contribute to some of the psychiatric symptoms apparent in these disorders, although further studies are needed to address this possibility.

Concluding remarks

In this review we have highlighted recent observations regarding the CNS migration and proliferation of microglia, and the subsequent physiological roles that these resident microglia mediate. In addition to their neuronal immune function in reacting to infections and clearing debris by phagocytosis, both resting and challenged microglia play an active role in neuronal circuit homeostasis. They contribute to PCD and synapse elimination during maturation of neuronal circuits and in response to neuronal injury. They also play a role in synapse and circuit homeostasis, by surveillance of synaptic elements in the adult brain and by actively participating in neurogenesis. Direct interactions between microglia and synaptic elements have been shown in uninjured brain, and some key molecules involved in these interactions include immune recognition molecules such as complement proteins. The ability of microglia to release ATP, neurotrophic factors, and cytokines underlies their capacity to modify neural circuits in physiological and pathological states. The microglia–synapse interactions during development and

Box 1. Outstanding questions

- Under what specific circumstances, and to what extent, can microglia be supplied to the brain from circulating monocytes? This question is important because it raises the possibility that circulating monocytes may be a novel pathway to deliver 'therapeutic microglia' to treat neurological and psychiatric disease.
- What are the signals and transduction mechanisms by which microglia communicate with developing neurons and adult neural progenitors to promote and/or regulate PCD?
- Do the same mechanisms mediate microglia–synapse interactions during developmental pruning of synapses, and during synaptic stripping following injury?
- What are the mechanisms and functional consequences of microglia surveillance of synapses in uninjured adult CNS? How do microglia choose a specific synapse to be eliminated, while others are spared?
- How do microglia sense the different levels of electrical activity of neuronal circuits with the necessary fine spatial resolution?
- Are the mechanisms by which microglia prune excitatory and inhibitory synapses the same? Do microglia selectively survey excitatory synapses in adult CNS and, if so, what may be the function of such a selective interaction?
- Does microglia dysfunction result in disease? Determining the mechanisms by which the known microglial hereditary pathologies result in their cognitive symptoms will shed light on possible broader contributions of microglial dysfunction to neurological disorders.

in adult homeostasis help to fine-tune neural circuits to optimize information processing and learning and memory. Such important physiological roles suggest that dysfunction of microglia may contribute to psychiatric and neurological disorders, and recent studies have identified hereditary disorders where microglial dysfunction has been implicated in disease pathogenesis. Such findings suggest that a paradigm shift is needed from considering microglia as only contributing indirectly to disease progression to potentially being actively involved in precipitating disease. However, much remains to be learned about the mechanisms and physiological consequences of microglia–synapse circuit interactions (Box 1). Addressing these issues will require new approaches for selective modification of microglia to reveal exactly how microglia affect neuronal activity, information processing, and disease pathogenesis.

Note added in proof

A recent paper by Li *et al.* [96] confirmed and extended in zebrafish larvae the characterisation of resting microglia-neuron contacts that has been described previously in mouse brain [65]. Microglial processes made frequent brief (5–6 mins) contacts with neuronal somata in the optic tectum of the larvae. Orientated movement towards neurons was activity-dependent and subsequent contacts were associated with an enlargment of the microglia process end into a bulbous tip. Both these steps were reduced when the small Rho GTPase Rac and neuronal pannexin channels were inhibited. Interestingly, microglia-neuron contacts resulted in a decrease of neuronal activity, suggesting a homeostatic role of microglia in identifying hyperactive neurons and then reducing their activity level.

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