Chapter 9
Developing and Mature Synapses

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Abstract Microglia are the resident immune cells of the brain. As such, they rapidly detect changes in normal brain homeostasis and accurately respond by fine-tuning in a tightly regulated manner their morphology, gene expression, and functional behaviour. Depending on the nature of these changes, microglia can thicken and retract their processes, proliferate and migrate, release numerous signalling factors and compounds influencing neuronal physiology (e.g., cytokines and trophic factors), in addition to secreting proteases able to transform the extracellular matrix, and phagocytosing various types of cellular debris, etc. Because microglia also transform rapidly (on a time scale of minutes) during experimental procedures, studying these very special cells requires methods that are specifically non-invasive. The development of such methods has provided unprecedented insights, these past few years, into the roles of microglia during normal physiological conditions. In particular, transcranial two-photon in vivo imaging revealed that presumably

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"resting" microglia continuously survey the brain parenchyma with their highly motile processes, in addition to modulating their structural and functional interactions with neuronal circuits along the changes in neuronal activity and behavioural experience occurring throughout the lifespan. In this chapter, we will describe how surveillant microglia interact with synaptic elements, and modulate the number, maturation, function, and plasticity of synapses in the healthy developing, mature, and aging brain, with consequences on neuronal activity, learning and memory, and the behavioural outcome.

Keywords Microglia • Synapses • Physiology • Neuronal circuit remodeling • Maturation • Function • Plasticity • Phagocytosis • Fractalkine • Complement • Brain-derived neurotrophic factor • Purinergic signalling

Bullet points

- Surveillant microglia dynamically interact with neuronal circuits at synapses.
- Axon terminals, dendritic spines, and peri-synaptic astrocytic processes are contacted in the healthy brain, during postnatal development, adolescence, adulthood, and aging.
- Microglia–synapse interactions are modulated by changes in neuronal activity and experience.
- Conversely, microglia contribute to regulating the number, maturation, function, and plasticity of excitatory synapses in normal physiological conditions.
- These new roles of microglia at synapses are mediated via phagocytosis, intercellular contacts, and release of soluble factors.
- The underlying molecular mechanisms discovered so far include fractalkine signalling, the classical complement pathway, brain-derived neurotrophic factor, DAP12, CD200R, and purinergic signalling.

9.1 Introduction

The capacity of the central nervous system (CNS) to adapt to the environment depends on the maintenance and refinement of neuronal circuits. This refinement is mainly achieved through the formation, elimination, or functional modulation of synapses, the elementary cellular components of neurotransmission. Because they have to transmit accurately the message from one neuron to another under all circumstances of life, synapses display strong plasticity, a phenomenon describing their ability to permanently adapt their structure and function to the demands of the circuitry. Synaptic plasticity comprises a vast repertoire of structural (number and shape of pre-synaptic axon terminals, post-synaptic dendritic spines, etc.) and functional changes (pre-synaptic release of neurotransmitters, modification of post-synaptic

receptors behaviour, second messenger signalling, etc.) (Bhatt et al. 2009; Holtmaat and Svoboda 2009; Bourne and Harris 2012; Choquet and Triller 2013).

Such precisely coordinated processes do not only require a synergistic choreography at the molecular, cellular, and behavioural levels, but also engage all the various cell types making up the CNS, including the supporting glial cells. The involvement of astrocytes with synapses has long been investigated (Nedergaard 1994; Parpura et al. 1994), and a large number of studies have revealed several aspects of their roles in the regulation of synapse formation, maturation, function, and plasticity (Chung et al. 2013; Clarke and Barres 2013; Pannasch and Rouach 2013). However, microglia are still an understated type of cell in normal CNS physiology, mostly studied in the context of immune and inflammatory responses since their first description by Pio del Rio-Hortega (Prinz and Priller 2014) (see Chap. 2 for further reading.)

As the primary immune effectors cells of the CNS, microglia are accurately equipped to sense and respond to changes in their local environment, associated with neuronal activity, experience, injury, or disease. Microglia express a vast repertoire of membrane receptors for various informative molecules such as neurotransmitters and neuromodulators, pro- and anti-inflammatory cytokines, growth factors, or extracellular matrix (ECM) components. (see Chaps. 3 and 4 for further reading.) These signalling molecules induce a constellation of intracellular responses in microglia, leading to concerted changes in morphology, gene expression, and functional dynamics (Bessis et al. 2007; Biber et al. 2007; Pocock and Kettenmann 2007; Kettenmann et al. 2011). As macrophages or phagocytes in residence into the CNS, microglia also display the ability to migrate and scavenge unwanted cells, cellular components, and debris by active phagocytosis (Gomez Perdiguero et al. 2013; Sierra et al. 2013). This ability is further refined by the extraordinary dynamics of microglial processes frequently interacting with the neuronal circuitry, particularly at synapses. Recently, microglial capacity to eliminate synaptic elements in an activity- and experience-dependent manner, and to promote the formation of dendritic spines, as well as modulate the maturation, function, and plasticity of excitatory synapses was additionally revealed in the developing and mature healthy brain (reviewed in Tremblay 2011; Bechade et al. 2013; Kettenmann et al. 2013; Schafer et al. 2013; Wake et al. 2013).

The combined evidence further advances the view that complex brain functions such as learning and memory emerge from the intercellular relationships between all types of cells in the CNS, acting in concert to achieve the most effective information processing and decision making. This chapter will describe the structural and functional interactions between microglia and synapses in the healthy brain, bridging the cellular/dynamic, ultrastructural and molecular levels, and summarize the key points that emerge from our current understanding of these roles, as well as the most promising directions to pursue in this nascent field of investigation.

9.2 Microglia–Synapse Interactions

Immunocytochemical electron microscopy (immune-EM; Tremblay et al. 2010) surprisingly revealed that almost all microglial processes (94 %) directly juxtapose synaptic elements in the visual cortex of adolescent mice, under physiological conditions. In particular, axon terminals, dendritic spines, peri-synaptic astrocytic processes, and synaptic clefts are contacted, in decreasing order of frequency (Tremblay et al. 2010). More recently, similar interactions were described in the visual and auditory cortex throughout adulthood and normal aging (Tremblay et al. 2012). Conversely, the proportion of each of those synaptic elements contacted by microglial processes could not be determined, since only a subset were stained for the microglial marker "ionized calcium-binding adapter molecule 1" (Iba1) and thus positively identified under these stringent immunocytochemical conditions. In the original characterization, serial section EM with 3D reconstruction showed that a single microglial process contacts multiple synaptic elements at multiple synapses simultaneously, sometimes with morphological specializations such as finger-like protrusions wrapping around dendritic spines and axon terminals (Fig. 9.1a,b). EM also revealed that microglial cell bodies and processes are distinctively surrounded by pockets of extracellular space, varying in volume by two orders of magnitude, in the cerebral cortex of adolescent and adult mice (Tremblay et al. 2010, 2012) (Fig. 9.1a,b). These pockets could result from the release of ECM proteases remodeling the extracellular space, such as metalloproteinases, plasminogens, and cathepsins, which might facilitate microglial dynamics, but also the motility, formation, and elimination of dendritic spines during normal physiological conditions (Tremblay 2011). Clathrin-coated pits were lastly encountered inside microglial processes and synaptic elements, specifically at their sites of contact (Tremblay et al. 2010), thus suggesting reciprocal exchange of molecular signals through clathrin-mediated endocytosis of membrane-bound receptors and their ligands (see Le Roy and Wrana 2005 for details on clathrin-mediated endocytosis). Therefore, these observations suggested that microglia can interact functionally with synaptic structures in the healthy brain (Tremblay and Majewska 2011).

Microglial contacts with subsets of axon terminals and dendritic spines were also encountered in vivo using non-invasive transcranial two-photon imaging. (see Chap. 4 for further reading on the technique.) To this end, Iba1$^{GFP/GFP}$ mice, in which GFP expression is driven by the Iba1 promoter (Hirasawa et al. 2005), were first crossed with Thy1-GFP mice (Feng et al. 2000) to visualize microglia and a subset of neuronal elements in the same color (green), including distal processes, dendritic spines, and axon terminals (Wake et al. 2009). Subsequently, the CX3CR1$^{GFP/-}$ mice in which the fractalkine receptor CX3CR1 only expressed by microglia in the healthy brain is replaced by the GFP reporter (Jung et al. 2000) were crossed with Thy1-YFP mice (Feng et al. 2000), thus providing an exceptional visualization of microglia and a subset of neuronal elements in two different colors (Tremblay et al. 2010). The CX3CR1 $^{GFP/-}$ heterozygous mice are partially deficient in fractalkine signalling (see discussion below), but microglial morphology, dynamic behaviour (Davalos et al. 2005; Nimmerjahn et al. 2005; Wake et al. 2009), dendritic spine turnover (Parkhurst et al. 2013), and microglial interactions with synaptic elements

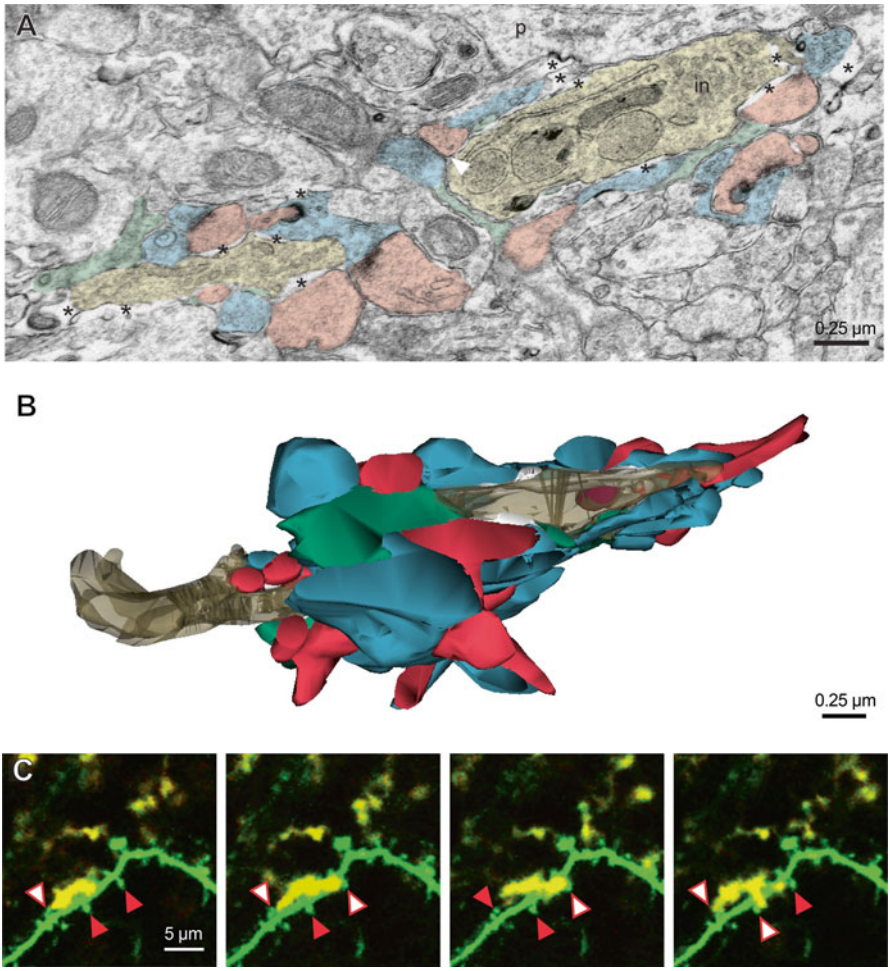

Fig. 9.1 *Microglia–synapse interactions in the healthy brain*. (**a**) Electron micrograph from a series of sections showing a proximal microglial process (cut in transverse, coloured in *yellow*) with a distal protrusion (cut longitudinally) contiguous to a neuronal perikaryon (p), and making direct contacts with dendritic spines (*pink*), axon terminals (*blue*), synaptic cleft (*arrowhead*), and peri-synaptic astrocytic processes (*green*) at postnatal day (P)28. Note the extracellular space pockets surrounding the microglia (*asterisks*). (**b**) Serial section 3D reconstruction of the microglial process and protrusion shown in **a**, uncovering simultaneous contacts with axon terminals (*blue*), dendritic spines (*red*), and peri-synaptic astrocytic processes (*green*) at multiple excitatory synapses. Extracellular space pockets are displayed in *white*, and a phagocytic inclusion within the proximal microglial process is in *purple*. (**c**) Two-photon time-lapse micrographs showing a dynamic microglial process (*yellow*) transiently interacting with dendritic spines (*green*) over the course of 20 min in the visual cortex of a CX3CR1$^{GFP/-}$/Thy1-YFP adolescent mouse. Each frame was captured 5 min apart. *Red arrowheads* indicate non-targeted dendritic spines, and *white arrowheads* targeted ones. Reproduced from Tremblay et al. (2010)

(Wake et al. 2009; Tremblay et al. 2010) were comparable in vivo between these CX3CR1$^{GFP/-}$ and the Iba1-GFP mice in which the CX3CR1 locus is intact. Using these mice, microglial processes were shown to frequently interact with axon terminals and dendritic spines, from the labeled subset of layer V pyramidal neurons, for durations varying between 5 and 30 min in the somatosensory and visual cortices during adolescence and adulthood (Wake et al. 2009; Tremblay et al. 2010) (Fig. 9.1c). Among these synaptic structures, small dendritic spines which are the most structurally dynamic and transient synaptic structures in mouse visual, somatosensory, motor, and frontal cortices in vivo (Trachtenberg et al. 2002; Zuo et al. 2005; Majewska and Sur 2006; Alvarez and Sabatini 2007; Holtmaat and Svoboda 2009) were preferentially targeted by microglial processes, and more frequently eliminated over 2 days than the non-contacted spines as revealed by chronic imaging of the same dendrites (Tremblay et al. 2010), raising the previously unsuspected possibility that microglia could be involved in their elimination. Both in vivo and in situ, microglia–synapse interactions were also found to be modulated by the ongoing changes in neuronal activity and sensory experience. Enucleation of the eyes, or injection of the sodium channel blocker tetrodotoxin (TTX) into the eyes, which silences excitatory and inhibitory neuronal activity, similarly reduced the frequency of microglial contacts with axon terminals in the primary visual cortex in vivo (Wake et al. 2009). Housing the animals in complete darkness for 1 week, from the beginning to the peak of the critical period for visual development, followed by their reexposure to normal circadian daylight for 2 days, a paradigm which increases the motility and elimination of dendritic spines (Majewska and Sur 2003; Keck et al. 2008), also reduced microglial dynamics in vivo, while increasing their perimeter of contact with synaptic elements and their association with the extracellular space in situ in the primary visual cortex of adolescent mice (Tremblay et al. 2010).

Further insight into this hypothesis that microglia may be responsible for eliminating synapses in the absence of pathological insults comes from ultrastructural observations. Immune-EM indeed revealed the presence of phagocytic inclusions positively identified as axon terminals and dendritic spines, based on their ultrastructural features (i.e., synaptic vesicles, post-synaptic densities) or immunostaining for specific markers, inside the cytoplasm and lysosomal compartments of microglial cell bodies and processes, during postnatal development, adolescence, adulthood, and normal aging, in mouse hippocampus, visual cortex and thalamus, and auditory cortex (Tremblay et al. 2010; Paolicelli et al. 2011; Schafer et al. 2012; Tremblay et al. 2012) (see Fig. 9.2 for examples and discussion below).

Fig. 9.2 (continued) t, axon terminal. Scale bars = 250 nm. Reproduced from Tremblay et al. (2010). (**c**) Example of microglial phagocytosis of synaptic elements during age-associated loss of sensory function. The immunostained microglial cell body contains a single cellular inclusion resembling an axon terminal "t" with clearly visible synaptic vesicles (inset) in the visual cortex of a 20-month-old mouse impaired in vision. Annotations as in **a**,**b**. Scale bars: 1 μm. Reproduced from Tremblay et al. (2012). (**d**) Microglial cell bodies and processes showing reduced engulfment of retinogeniculate projections during early postnatal development, in mice deficient in the complement opsonin C1q or retinal transforming growth factor β (TGF-β) signalling. Volume of individual cells and engulfed cholera toxin-β conjugated to Alexa 488 or Alexa 594 (CTB-488 or CTB-594) injected into the left and right eyes, respectively, to label retinal projections at P10. Reproduced from Bialas and Stevens (2013)

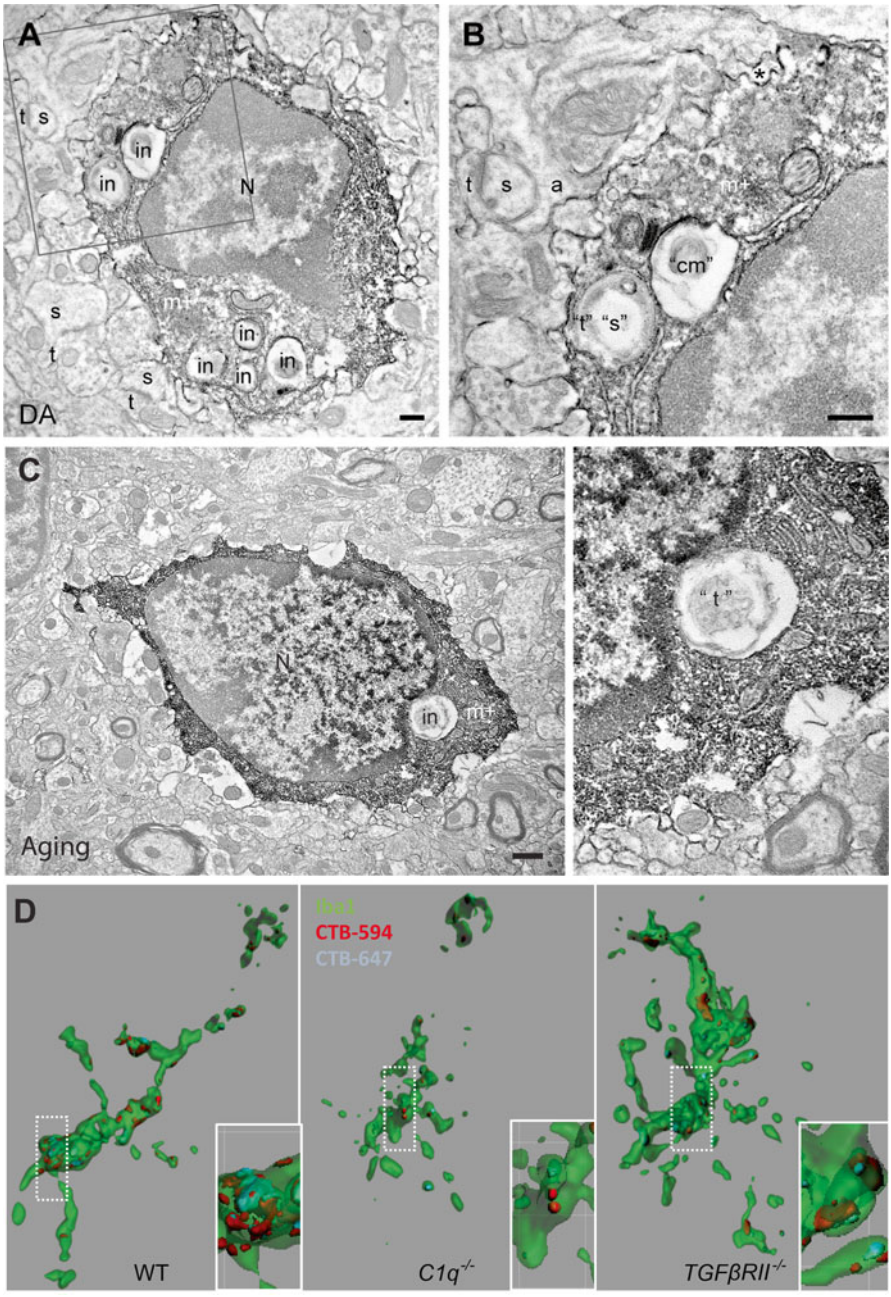

Fig. 9.2 *Microglial engulfment of synaptic elements during normal physiological conditions*. (**a**,**b**) Examples during dark-adaptation (DA), a form of visual plasticity that is induced by housing the animals in complete darkness for 1 week, from the beginning to the peak of the critical period in the visual system, i.e., postnatal day (P)21–28. In A, an Iba1-immunopositive microglial cell body (m+) observed in the visual cortex displays multiple cellular inclusions (in) at P28. (**b**) shows a magnified view of the boxed region in (**a**). One of these inclusions resembles a dendritic spine (s) receiving a synapse from an axon terminal (t), while the other inclusion contains an accumulation of cellular membranes probably undergoing digestion (cm). a, peri-synaptic astrocyte; *N*, nucleus; s, dendritic spine;

The phagocytosed elements displayed ultrastructural features of healthy cells such as an electron-lucent cytoplasm, contrarily to the apoptotic elements which are phagocytosed during injury or disease (Schmechel 1999), and the apoptotic newborn cells which are phagocytosed during adult neurogenesis (Sierra et al. 2010). Microglial processes with phagocytic specializations were also observed in vivo, sometimes encircling neuronal elements, but since these structures were stable throughout the 30–120 min imaging sessions, episodes of microglial engulfment followed by a full disappearance of the synaptic elements were not completely visualized. Both in situ and in vivo, microglial phagocytic structures became more prevalent during light deprivation, and still persisted after reexposure to light (Tremblay et al. 2010) (Fig. 9.2a,b), suggesting a role in the refinement of neuronal circuits during adaptation to a novel environment. More recently, phagocytic inclusions within microglial cell bodies and processes were also observed during adulthood and normal aging. Immune-EM revealed that microglial cell bodies and processes accumulate large amounts of phagocytic inclusions, sometimes with ultrastructural features of axon terminals and dendritic spines, with or without an electron-lucent cytoplasm, but also lysosomal lipopigments, large vesicles, vacuoles, and lipid droplets, in the visual and auditory cortices of two stains of mice normally undergoing complementary age-related loss of vision (CBA/CaJ mice) or hearing (C57Bl/6 J mice) during their second year of life (Fig. 9.2b). This accumulation was exacerbated by the age-related loss of visual or auditory function, with nearly all microglia containing phagocytic inclusions and ~20 % of microglial cells becoming almost completely filled by the inclusions (Tremblay et al. 2012). Together, these observations during adolescence, adulthood, and normal aging suggested that microglial phagocytosis is regulated in an experience-dependent manner in the mature CNS, thus proposing a physiological role for microglia–synapse interactions in the refinement of neuronal circuits throughout the lifespan.

9.3 Regulating Synapse Number

At this early stage of investigation in the field, the molecular mechanisms regulating microglial interactions with synapses, as much as their functional consequences on the brain and behaviour, still remained undetermined. Three main investigations have recently started to address these questions, in situ and in vivo, showing the involvement of fractalkine signalling and the classical complement cascade in regulating microglial pruning of synapses during early postnatal development, and the role of brain-derived neurotrophic factor (BDNF) in promoting the formation of dendritic spines during adolescence and adulthood, as discussed below.

9.3.1 Fractalkine Signalling

The first evidence that microglia–synapse interactions have functional consequences comes from early postnatal development, a period of remarkable plasticity where the remodeling of neuronal circuits is particularly exacerbated. The maturing neurons actively extend supernumerary connections that are progressively removed while others are maintained and strengthened. The term "pruning" refers to a selective developmental process regulated by neuronal activity that involves both large-scale elimination of axons and dendritic arbours, as well as the local removal of axon terminals or dendritic spines without the death of parent neurons (Herrmann and Shatz 1995; Tessier-Lavigne and Goodman 1996; Hua and Smith 2004). The large-scale removal of neuronal processes was initially proposed to be mediated by degeneration, cytoskeletal breakdown, fragmentation, and/or autophagy, while the pruning of synapses was considered to result from cytoskeletal collapse leading to retraction of the synaptic structure (Luo and O'Leary 2005), through coordinated interactions with the ECM and peri-synaptic astrocytic processes (Ethell and Pasquale 2005; Haber et al. 2006; Majewska and Sur 2006; Hotulainen and Hoogenraad 2010). Nevertheless, the cellular and molecular mechanisms that determine whether and how particular subsets of synapses are specifically eliminated remain an important question in the field, and the involvement of microglia in this process has just begun to be unravelled.

Analyses of mice bearing loss-of-function mutations in microglial genes encoding signalling molecules are useful for hinting at possible links between microglial function and neuronal activity. One example of such molecules involved in neuron-microglia communication is fractalkine, a member of the ∂-chemokine subfamily also known as neurotactin or CX3CL1. Fractalkine is expressed almost exclusively by neurons (Tarozzo et al. 2003; Ransohoff 2009) and can be soluble or membrane-bound (Garton et al. 2001; Tsou et al. 2001), but the individual activities of these two forms still need to be clarified, especially in the brain. Most importantly, fractalkine has a unique receptor, CX3CR1, that is only expressed by microglial cells in the healthy brain (Combadiere et al. 1998; Cardona et al. 2006). The involvement of fractalkine signalling in mediating the elimination of synapses during normal development was particularly addressed by examining CX_3CR1-deficient mice in the CA1 region of the hippocampus during the first postnatal weeks, a period of intense synaptic remodeling. In this manner, Paolicelli and colleagues revealed a slower increase of microglial density in the $CX3CR1^{GFP/GFP}$ mice (or $CX3CR1^{KO/KO}$) versus $CX3CR1^{GFP/-}$ littermates between postnatal day (P)8 and 28. This delayed colonization of the maturing hippocampus (see Chaps. 7 and 8 for further reading on the roles of microglia during normal brain development) was found to be accompanied by a transient increase in dendritic spine density on the pyramidal neurons apical dendrites of $CX3CR1^{KO/KO}$ mice compared with wild-type littermates, over the same developmental period (Paolicelli et al. 2011). However, since microglial engulfment of dendritic spines was observed but not quantified, it remains to be elucidated whether fractalkine signalling instructs microglial recruitment to the proximity of

maturing dendrites or contributes more directly to regulating microglial phagocytosis of dendritic spines in the developing hippocampus.

In a follow-up study by the same group, it was recently shown that the CX3CR1$^{KO/KO}$ mice additionally display a lasting impairment of hippocampal synaptic connectivity into adolescence. In particular, quantifying at the ultrastructural level the density of multi-synaptic boutons, i.e., axon terminals contacting two dendritic spines, revealed a significant reduction in the knockout mice compared to wild-type littermates at P40 (Zhan et al. 2014) despite a normalized density of dendritic spines observed in adult animals (Paolicelli et al. 2011). Local field potentials (LFPs), which inform about the sum of synaptic activity within a particular volume, were also investigated at P40. As a measure of long-range connectivity, coherence spectra of the LFPs were calculated, under the premise that high coherence values would reflect strongly connected structures. By implanting electrodes in vivo within different brain regions simultaneously, a significant decrease in the coherence between the hippocampus and prefrontal cortex was found in adult CX3CR1 knockout mice, compared to wild-type littermates, thus reflecting a decrease in functional connectivity between these two areas. Supporting these results, the global connectivity assessed by functional magnetic resonance imaging (fMRI) was also significantly reduced across brain regions in the CX3CR1$^{KO/KO}$ mice, with the difference becoming particularly evident for distant regions (Zhan et al. 2014). These changes in neuronal circuits also seem to have behavioural repercussion, since social interactions were found to be altered in the CX3CR1$^{KO/KO}$ mice. In the same study by Zhan and colleagues, impaired social behaviour was particularly observed in juvenile mice, displaying no preference for their own mother over an inert stimulus, as assessed by the homing testing of motivation towards a relevant stimulus, i.e., the odour of the nest. However, no impairment in performing the novel object recognition test, which relies on prefrontal cortex function, was observed in the same mice. Similarly, adult CX3CR1$^{KO/KO}$ mice tested in a standard three-chamber apparatus failed to display significant interest towards a sex-matched social stimulus, compared to wild-type controls (Zhan et al. 2014). No deficit in responding to social olfactory cues was reported in these mice, suggesting that the impairment observed in social behaviour was due to reduced social motivation, rather than difficulties with the discrimination of social cues. Increased grooming behaviour was also reported in adult CX3CR1 knockout mice, when tested in a novel cage for 10 min, suggesting a propensity for increased repetitive behaviour, particularly triggered under stressful conditions (Zhan et al. 2014). Additionally, Rogers and colleagues have reported deficits in different forms of learning and memory in the adult CX3CR1$^{KO/KO}$ mice. Motor learning was found to be compromised in CX3CR1$^{KO/KO}$ mice versus wild-type littermates, using the rotarod test for balance, coordination, physical condition, and motor planning (Rogers et al. 2011). Locomotor and exploratory activity was, however, similar between genotypes, as assessed in the open field test, and no difference was observed in anxiety behaviour, measured by the elevated plus maze. Associative learning and memory was also found to be altered in a standard fear-conditioning paradigm, as the knockout mice failed to display reduced freezing (Rogers et al. 2011). The CX3CR1$^{KO/KO}$ were lastly impaired in the

water maze, further supporting a role for fractalkine signalling in modulating hippocampal-dependent learning and memory. These effects could be mediated by microglial release of the pro-inflammatory cytokine interleukine-1β (IL-1β) since intrahippocampal infusion of its antagonist IL-1ra significantly reversed the deficits in cognitive function measured in the knockouts (Rogers et al. 2011). Providing additional insights, Maggi and colleagues also revealed that the $CX3CR1^{KO/KO}$ mice failed to perform better in the water maze following prolonged exposure to an enriched environment, compared with standard housing conditions, even though they were not significantly different from aged-matched wild-type controls in their ability to learn the water maze task in this study (Maggi et al. 2011), an apparent discrepancy which warrants further investigation.

These recent findings are complementing one another in proposing a role for CX3CL1-CX3CR1 interactions in mediating the refinement of neuronal circuits, learning and memory, and the behaviour, as well as the maturation and plasticity of synapses as described in the following section. Since microglia are the only cells expressing CX3CR1 in the healthy brain, they might be crucially involved in all of these processes shown to be influenced by fractalkine signalling during normal physiological conditions (also see Paolicelli et al. 2014). Nevertheless, the microglial effector functions which are precisely recruited, the molecular mechanisms acting downstream of fractalkine signalling, and the respective contributions of soluble versus membrane-bound fractalkine remain to be elucidated.

9.3.2 Complement Proteins

Pruning has been classically described in the mouse retinogeniculate system (Huberman 2007, 2008; Hong and Chen 2011; Schafer et al. 2012). This system is comprised of retinal ganglion cells (RGCs) that project axons to eye-specific territories in the dorsal lateral geniculate nucleus (dLGN) of the thalamus. During early postnatal development, retinogeniculate synapses undergo activity-dependent pruning to achieve the precise connectivity characteristic of the adult system, including proper eye-specific segregation, as required for the development of a normal binocular vision (Chen and Regehr 2000; Hooks and Chen 2006; Huberman 2007, 2008).

In this system, ramified microglia were also revealed to be intimately associated with retinogeniculate synapses during active synaptic remodeling, which peaks around P5 in mice, thereby suggesting microglia as a candidate cellular mediator of synaptic pruning. To test this hypothesis, Schafer and colleagues developed an in vivo engulfment assay in order to determine whether microglia phagocytose retinogeniculate synapses (Schafer et al. 2012). Using the $CXCR1^{GFP/-}$ mice, eye-specific RGC axonal inputs were labelled by injecting Alexa-conjugated anterograde tracers (cholera toxin B) into the eyes of postnatal mice. High-resolution confocal microscopy revealed an internalization of the RGC axonal terminals within microglia throughout the dLGN (see Fig. 9.2c), which was particularly exacerbated at P5, suggesting its developmental regulation. Moreover, immune-EM analyses revealed

internalization of pre-synaptic elements contained within the microglial cytoplasm and lysosomal compartments. These observations proposed that microglia-mediated pruning could be actively involved during the peak of this early phase of synaptic remodelling in the retinogeniculate system, i.e., around P5, guiding the search for candidate molecules controlling this form of microglia–synapse interactions.

Complement proteins are a group of innate immune proteins associated with the rapid removal of apoptotic cells and pathogens in the periphery. C1q is the initiating protein of the classical complement cascade. When C1q binds to and coats (or opsonizes) dead cells, pathogens, or cellular debris, it triggers a protease cascade leading to the deposition of the downstream complement protein C3. Opsonization with the activated C3 fragments C3b can directly activate C3 receptors on macrophages and microglia, thereby triggering elimination by phagocytosis, or trigger the terminal activation of the complement cascade, leading to cellular lysis through the formation of a lytic membrane attack complex (Gasque 2004; van Lookeren Campagne et al. 2007). Recent studies using array tomography on 70 nm-thick brain sections revealed that the complement proteins C1q and C3 are widely expressed in the healthy developing LGN where they partially localize to excitatory synapses (Stevens et al. 2007). Mice deficient in C1q and C3 also have sustained defects in structural and functional elimination of synapses in the mouse developing visual system, raising questions about how complement-targeted synapses could be eliminated. Since microglia are the only resident brain cells to express CR3 in the healthy brain (Ransohoff and Perry 2009), these observations suggest C3-CR3-mediated phagocytosis as a potential mechanism underlying microglia-mediated pruning in the developing brain. Consistent with this hypothesis, mice deficient in C3 or CR3 resulted in a ≈50 % deficit in the ability of microglia to engulf RGC axons (Schafer et al. 2012). Moreover, the C3 and CR3 knockout mice have significantly more excitatory synapses, accompanied by defects in eye-specific segregation in the LGN at P32–P35, indicating that altered complement signalling early during development results in a sustained defect of synaptic connectivity into late adolescence (Schafer et al. 2012). In another recent study, the density of axon terminals on layer V pyramidal neurons was additionally found to be significantly increased in cortical slices derived from C1q knockout mice (P27–P31) and accompanied by epileptic seizures at the behavioural level, thus suggesting a failure to prune excitatory synapses during development (Chu et al. 2010).

What controls the timing and location of microglia-mediated synaptic refinement in the brain? Previous findings suggested that an astrocyte-derived factor triggers neuronal expression of C1q mRNA in purified postnatal retinal ganglion neurons in culture (Stevens et al. 2007). As the initiator of the classical complement cascade, C1q is a critical point of regulation in this pathway. C1q is developmentally expressed in postnatal RGCs, especially at P5, the peak of microglia-mediated pruning (Stevens et al. 2007). In the immune system, C1q can be modulated by rapid cytokine signalling pathways. A recent study identified transforming growth factor β (TGFβ) as a factor secreted by astrocytes that regulates C1q expression in RGCs (Bialas and Stevens 2013). This study showed that TGFβ is mostly expressed

by astrocytes in the retina during the refinement period and TGFβ receptors (TGFβRII) are developmentally expressed in the postnatal RGCs. Blocking TGFβ signalling in retinal neurons resulted in a significant reduction of C1q expression in postnatal RGCs as well as reduced synaptic localization of complement in the dLGN in situ. Moreover, specific disruption of TGFβRII in retinal neurons, using retina-specific TGFβRII knockout mice generated using a Cre recombinase approach under the Chx10 promoter, which is active at embryonic day 13.5 in the outer neuroblastic layer of the retina only, also inhibited complement and microglia-mediated synaptic pruning in the dLGN (Fig. 9.2c) (Bialas and Stevens 2013). This data suggested that TGFβ-dependent regulation of neuronal C1q in the retina regulates downstream complement-dependent synapse elimination, a novel role for the TGFβ cytokine-signalling pathway in the regulation of microglia-mediated synaptic refinement in the postnatal brain.

Another important regulator of microglia-mediated pruning is neuronal activity. Synapse elimination is thought to result from competition between neighboring axons for territory on a post-synaptic neuron based on differences in patterns or levels of neuronal activity (Shatz 1990; Arnold 1999; Sanes and Lichtman 1999; Huberman et al. 2008). In the retina, spontaneous neuronal activity from both eyes is thought to drive eye-specific segregation and retinogeniculate pruning (Stellwagen and Shatz 2002; Torborg and Feller 2005; Huberman 2007). While the specific properties of retinal activity that guide this process remain elusive, these findings are consistent with a model in which left and right eye retinal axons compete for territory on post-synaptic dLGN relay neurons. Consistent with this idea, recent studies reveal that microglia-mediated pruning of RGC inputs in vivo is an activity-dependent process (Schafer et al. 2012). When competition between RGC inputs originating from the two eyes was enhanced by monocular injection of TTX or forskolin, an activator of adenylate cyclase which raises the level of cyclic AMP thereby increasing spontaneous neuronal activity, microglia preferentially engulfed inputs from the eye with reduced neuronal activity relative to the other eye, leading to an impairment of eye-specific segregation as observed in the C3- and C3R-deficient mice (Schafer et al. 2012). These data are consistent with previous work demonstrating a decreased synaptic territory of the "weaker" inputs and increased territory of "stronger" inputs within the dLGN (Penn et al. 1998; Cook et al. 1999; Stellwagen and Shatz 2002; Del Rio and Feller 2006; Hooks and Chen 2006; Huberman et al. 2008). Although it is not yet known how microglia specifically target the "weaker" synapses, complement is indeed partially required for microglial engulfment, suggesting a possible link between neuronal activity and the complement cascade. Together, these findings suggest a model in which context-dependent molecular cues (i.e., TGFβ) and neuronal activity cooperatively regulate the timing and degree of microglia-mediated pruning during development in the retinogeniculate system. Whether these mechanisms apply to other developmental or adult pruning conditions in health or disease remains to be determined.

9.3.3 Brain-Derived Neurotrophic Factor

Beyond early postnatal development, the underlying molecular mechanisms and functional consequences of microglia–synapse interactions were recently investigated during adolescence and early adulthood. The role of microglial BDNF, a potent regulator of synaptic development and plasticity that is expressed by various cell types including microglia (Chao 2003; Chakravarthy et al. 2006), was particularly examined. Microglial release of BDNF has also been shown to modulate neuronal plasticity in a mouse model of neuropathic pain (see Chap. 11 for further reading.) (Coull et al. 2005). In this study, the BDNF locus was deleted in microglia specifically, following early postnatal development, using CX3CR1-CreER crossed with BDNF-floxed mice treated with tamoxifen to induce BDNF deletion during adolescence. The mice were further crossed with Thy1-YFP mice to visualise neurons and analyse the turnover of dendritic spines (i.e., rates of formation and elimination) using chronic two photon in vivo imaging over 2 days (Parkhurst et al. 2013). This analysis revealed that removing microglial BDNF induces a significant decrease in the formation rate of dendritic spines (i.e., proportion of dendritic spines only observed in the second imaging session) in the motor cortex during learning of the rotarod, leaving unchanged the elimination of dendritic spines (i.e., proportion of dendritic spines only observed in the first imaging session) (Parkhurst et al. 2013). These observations thus revealed an unexpected role for microglia in spinogenesis and possibly in the formation of synapses in the mature healthy brain.

Similar findings were obtained in a microglial depletion model achieved by crossing the CX3CR1-CreER with diphteria toxin receptor (DTR)-floxed mice. These mice were treated with tamoxifen shortly after birth to induce DTR expression, followed by diphtheria toxin (DT) administered 7 days before imaging performed at P19 or P30. This time frame enabled to deplete microglial populations while preventing the brain replenishment by peripheral immune cells. In this manner, microglial depletion caused a ≈50 % decrease in the elimination rate of spines, measured over 4 days, at P19, and a ≈20 % decrease at P30. Even though microglia–synapse interactions were not visualised, these observations confirm microglial contribution to synaptic pruning in the healthy mature brain. In these microglia-depleted mice, a decreased formation and elimination of dendritic spines were also observed during motor learning on the rotarod. The difference between the microglia-depleted mice and non-depleted controls was significant for both the formation and elimination of dendritic spines at P30, and only for the formation of dendritic spines at P60 (Parkhurst et al. 2013). These observations suggest that the developmental regulation of these processes could follow a different time course, or that different mechanisms could be recruited depending on the stage of the lifespan. Providing additional mechanistic insights, in vitro experiments were additionally performed, showing that media from cultures of purified microglia (microglia-conditioned media) phosphorylates the BDNF tyrosine kinase receptor TrkB in cultures of purified cortical neurons (Parkhurst et al. 2013). Since the autophosphorylation of TrkB is required for its involvement in synaptic plasticity (Chao 2003), these observations suggest

that microglial BDNF induces functional signalling in the neurons to induce dendritic spine formation.

At the behavioural level, microglial BDNF removal and microglial depletion were both accompanied by an impairment of motor learning on the rotarod. Auditory fear conditioning mainly relying on hippocampal, amygdala, and auditory cortex function was also impaired in both types of mice compared to wild-type mice, but there were no significant deficits of novel object recognition, a paradigm which depends on the prefrontal cortex, in the microglial BDNF-deficient mice contrarily to the microglia-depleted mice (Parkhurst et al. 2013). These findings suggest that different cellular and molecular mechanisms could be recruited depending on the particular form of memory formation and learning that is involved. The overall findings also underscore an important role of microglia during learning within multiple brain regions, particularly including the motor cortex, auditory cortex, the hippocampus, and amygdala which are together part of the limbic system, and the prefrontal cortex. However, more recent data found that dietary administration of a selective inhibitor of the colony-stimulating factor 1 receptor (CSF1R), which is exclusively expressed by microglia in the healthy brain, also depletes microglia during adulthood, possibly through a reduction of their survival. Noteworthy, depleting microglia with this pharmacological approach does not induce deficit in various forms of learning and memory tasks, including the rotarod (Elmore et al. 2014), suggesting that the method of microglial elimination could bias the interpretation of data. Future work will certainly elucidate this apparent paradox.

From the combined observations, a "two-step model" could be hypothesized, according to which soluble fractalkine would first mediate microglial recruitment, promoting their migration and specific interactions with the relevant neuronal circuits requiring function intervention, where fractalkine is up-regulated to "call" for microglial support during development or learning. According to this model, microglia would first address their terminal processes expressing CX3CR1 to the proximity of membrane-bound fractalkine, to ensure proper contact with neuronal branches and synaptic extremities. Once properly positioned in these hot-calling-spots, microglia would then respond to specific molecular cues such as complement molecules (e.g., C3) selectively targeting the weak and inappropriate synaptic terminals for pruning, or additional and yet unidentified cues specifically dictating the release of BDNF to promote dendritic spine formation.

9.3.4 Overview, Open Questions, and Further Insights

Several studies have now demonstrated that microglia play an active role in mediating both the formation and elimination of synaptic structures (see Fig. 9.3 for a schematic overview of the cellular and molecular mechanisms recruited). These studies have thereby opened many new questions that will certainly be tackled in the future. For instance, do microglia interact with inhibitory synapses during normal physiological conditions? Up to now, microglial-related pruning has only been

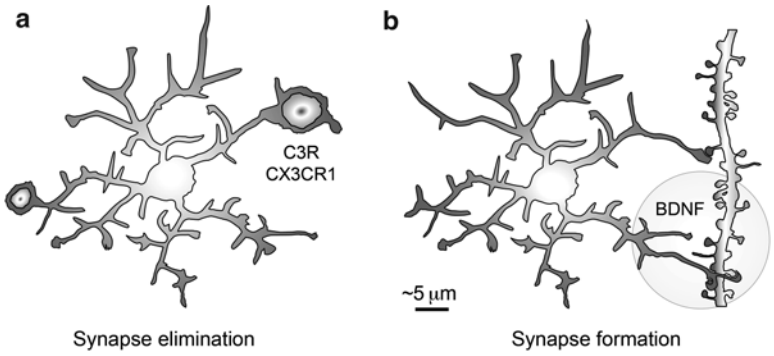

Fig. 9.3 *Microglial regulation of synapse number: an overview of the proposed mechanisms.* (**a**) Microglial pruning of synaptic elements is mediated by the classical complement pathway in the developing retinogeniculate system. In particular, mice deficient in the opsonin C3 or microglial C3 receptor (C3R) displayed a similar impairment in the eye-specific segregation of retinal inputs to the visual thalamus, accompanied by a reduced microglial engulfment of their projections. Additionally, fractalkine signalling could also be involved in mediating microglial pruning of synapses in the hippocampus during early postnatal development, since a reduced microglial colonisation of the hippocampus accompanied by an increased density of dendritic spines has been observed in mice deficient in the fractalkine receptor, CX3CR1, only expressed by microglia in the healthy brain, compared with wild-type littermates. Evidence of reduced synaptic multiplicity (i.e., density of axon terminals contacting two dendritic spines), impairment of synaptic maturation, function and plasticity, learning and memory, and social behaviour were also reported in the CX3CR1 knockouts. (**b**) Microglial release of brain-derived neurotrophic factor (BDNF) was also recently revealed to promote dendritic spine formation during motor learning, in the primary motor cortex

demonstrated for excitatory synapses. Yet, the frequency of miniature inhibitory post-synaptic currents (mIPSCs) has been shown to be reduced in CX3CR1$^{KO/KO}$ mouse hippocampus at P15 (Zhan et al. 2014), indicating a possible functional relationship between microglia and inhibitory synapses. These mIPSCs were recorded from interneurons in the presence of the sodium channel blocker TTX which prevents action potential-induced currents, thus enabling to measure the post-synaptic currents induced by the random release of synaptic vesicles. However, further studies are needed to elucidate whether inhibitory synapses, for example, can serve as a substrate for microglial-mediated pruning. What are the regional and sex-specific differences in microglial involvement with synapses? Complement-deficient animals display more synapses in the visual system (Schafer et al. 2012), but not in the spinal cord (Lobsiger et al. 2013), suggesting tissue-specific action of the complement-dependent pruning. Recently, intracerebroventricular injection of the anti-inflammatory antibiotic minocycline, which is commonly used for preventing microglial activation but also acts on astrocytes and other inflammatory cells, was shown to prevent the occurrence of sex differences in microglia (density, morphology), and the masculinization of dendritic spine density in the preoptic area of the hypothalamus during the critical period for sexual differentiation, i.e., between P0 and P3, with consequences on the adult copulatory behaviour. In this

context, microglial regulation of estradiol (aromatized locally from testis-derived testosterone) and prostaglandin levels was proposed as an underlying mechanism (Lenz et al. 2013).

What are the molecular cues which determine microglial recognition and elimination of synaptic elements specifically, i.e., versus other neuronal compartments such as axons and dendrites? Microglial processes sometimes contact dendrites and axons in vivo and in situ, but their consequences still remain undetermined. What are the molecular mechanisms responsible for microglial recognition and elimination of particular subsets of synaptic elements at particular excitatory synapses? Linnartz and colleagues have recently revealed that changes in the neuronal glycocalyx induce activation of the classical complement cascade in vitro. In this study, desialylated (i.e., lacking the terminal sugar residue sialic acid) neurites from hippocampal neurons were found to be preferentially phagocytosed by hippocampal microglial cells in primary coculture via a CR3-dependent mechanism (Linnartz et al. 2012). Do classical mechanisms such as cytoskeletal collapse accompanied by dendritic spine retraction compete or cooperate with microglial involvement in the formation and elimination of synaptic elements? How do microglia cooperate with astrocytes and other types of glial cells in mediating these structural changes at synapses? Future works might unravel novel molecular pathways regulating the refinement of neuronal circuits in the healthy brain and will also determine the relationships between fractalkine, the complement pathway, and BDNF and elucidate the functional relevance and contextual specificities of each of these pathways.

9.4 Regulating Synapse Maturation, Function, and Plasticity

As cells of the immune system, microglia are able to sense and react to modifications of their environment and to physiologically interact with their neighboring cells. Recent studies revealed that microglia functionally communicate with neurons and have the capacity to regulate the maturation, function, and plasticity of synapses, as will be discussed in this section.

9.4.1 Maturation of Synapses

In addition to impairing the elimination of synapses, the brain functional connectivity, learning and memory, and different types of behaviour, removing microglial CX3CR1 has been shown to delay the maturation of excitatory synapses in hippocampal slices. During early postnatal development, the ratio between the amplitude of spontaneous excitatory post-synaptic currents (sEPSC) and the amplitude of miniature (mEPSCs) recorded from hippocampal pyramidal cells progressively increases as neuronal circuits mature (Hsia et al. 1998). Since mEPSCs are recorded in the presence of the sodium channel blocker TTX, they enable to measure the

post-synaptic currents induced by the random release of synaptic vesicles. On the other hand, sEPSCs reflect currents which are triggered by an action potential, thus measuring synaptic efficiency. The amplitude of sEPSC normally increases over the course of postnatal development, as functional synapses increase in number and neuronal networks become properly interconnected (Hsia et al. 1998). On the contrary, the amplitude of mEPSCs remains relatively constant across the same developmental period. In hippocampal slices of CX3CR1$^{KO/KO}$ mice compared to wild-type littermates, this sEPSC/mEPSC ratio was shown to be reduced at P15 as well as at P40, thus suggesting a deficit in the maturation of synaptic connectivity (Paolicelli et al. 2011; Zhan et al. 2014). This observation raised the provocative idea that microglia could regulate the maturation of synapses.

Such a role in synaptic maturation is supported by the recent observations of Hoshiko and colleagues, revealing that microglial recruitment to the sites of maturating synapses begins at P5 in the somatosensory cortex and is mediated by fractalkine signalling (Hoshiko et al. 2012). In this study, a reduced density of microglial cells was observed in the thalamocortical clusters, at P6 and P7, but not at P9, in the CX3CR1$^{KO/KO}$ mice versus wild-type littermates. This reduced microglial density inside the clusters was accompanied by an increased density outside the clusters, resulting in an unchanged overall density within the somatosensory cortex. Interestingly, this delayed migration of microglial cells was also paralleled by a transient impairment in the maturation of synaptic properties (Hoshiko et al. 2012). At thalamocortical synapses, the composition of NMDA receptors (NMDARs; named after their agonist *N*-methyl-D-aspartate), which are the main glutamate receptors in the CNS, is known to switch from GluN2B to GluN2A subunits between the first and second postnatal weeks. In this study by Hoshiko and colleagues, a higher proportion of GluN2B-containing NMDARs was reported in the CX3CR1 knockout mice, associated with slower kinetics of the NMDA-mediated synaptic currents. Indeed, the decay time of the NMDAR-mediated responses was found to be significantly reduced at P9–P10 using single-cell recordings in the CX3CR$^{KO/KO}$ mice compared to wild-type littermates. However, the defect was transient, without any difference in the decay time of NMDAR-mediated responses remaining at P27–P33 (Hoshiko et al. 2012). To mediate this delayed maturation of thalamocortical synapses, microglia have been proposed to influence the function of glutamate receptors by their release of signalling factors which remain to be identified and might include IL-1β, tumour necrosis factor α (TNFα), and BDNF, which are known for modulating the expression and function of glutamate receptors in vitro (Beattie et al. 2002; Chao 2003; Zhang et al. 2010; Zhong et al. 2010).

9.4.2 Function and Plasticity of Synapses

Long-term potentiation (LTP) is a long-lasting enhancement of synaptic transmission resulting from strong stimulation of the same synapses. This is the most widely used cellular paradigm of synaptic plasticity. Synaptic alterations including

enhanced hippocampal LTP in adolescent brain slices (Roumier et al. 2004, 2008) and impairment of higher brain functions such as loss of memory, aphasia, agraphia, acalculia, and apraxia (Paloneva et al. 2000) have also been demonstrated upon the loss-of-function mutation of DAP12, a transmembrane signalling protein that is exclusively expressed by microglia in the healthy brain (Roumier et al. 2004). Another microglial protein which was shown to be involved in synaptic regulation is CD200R, a membrane protein exclusively expressed by microglia in the CNS during normal physiological conditions that interacts with CD200 expressed by neurons, oligodendrocytes, and astrocytes (Costello et al. 2011). It has been demonstrated that LTP is inhibited in hippocampal slices from adult CD200-deficient mice, further supporting the notion that the integrity of microglial signalling is important for neurotransmission homeostasis (Costello et al. 2011). Finally, hippocampal LTP in the adult was shown to be reduced (Maggi et al. 2009; Rogers et al. 2011) or increased (Maggi et al. 2011) in $CX3CR1^{KO/KO}$ mice depending on the study. Despite the disagreement warranting further investigation, this observation that LTP is modified in CX3CR1-deficient mice suggests that microglia could be involved in regulating synaptic function and plasticity. Paolicelli and colleagues lastly examined the effects of fractalkine signalling on the induction of long-term depression (LTD), another form of synaptic plasticity reducing synaptic transmission on the opposite of LTP, in hippocampal slices from the CX3CR1 knockout mice and their wild-type littermates, reporting no difference between genotypes at P40, despite a significant enhancement at P13 (Paolicelli et al. 2011), thus suggesting that in the mature healthy brain fractalkine signalling selectively modulates LTP. These examples only show correlations between the alterations of microglial signalling and synaptic dysfunction and indirect developmental or systemic issues cannot be excluded in gene knockout animals. However, the recent work of Parkhurst and colleagues demonstrated that genetic depletion of microglia in adult mice induces a robust decrease in the frequency of mEPSCs mediated both by NMDARs and AMPA receptors (AMPARs, named after their agonist α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) (Parkhurst et al. 2013), probably revealing a direct regulation of glutamatergic transmission by microglia.

Several mechanisms have now been identified showing that microglia actively influence the synaptic properties in hippocampal slices. For instance, upon stimulation with the nucleotide ATP, cultured microglia can rapidly shed micro-vesicles, most probably by a mechanism depending on the P2X7 purinergic receptor (Bianco et al. 2005). When these vesicles were applied to cultured hippocampal neurons, they increased the frequency of mEPSCs (Antonucci et al. 2012), raising the hypothesis that neurotransmission could be regulated by physical contacts. Microglia produce a broad spectrum of signalling molecules, from cytokines to neurotransmitters and ECM proteins known to regulate the synaptic function (reviewed in Bechade et al. 2013). In line with such production, several studies have demonstrated a regulation of synaptic properties by microglial signalling molecules. Stimulation of microglia by fractalkine in neuronal cultures was shown to induce a strong and rapid modulation of AMPARs-mediated calcium currents in neurons (Meucci et al. 1998). This modulation has also been confirmed in acute hippocampal slices, in

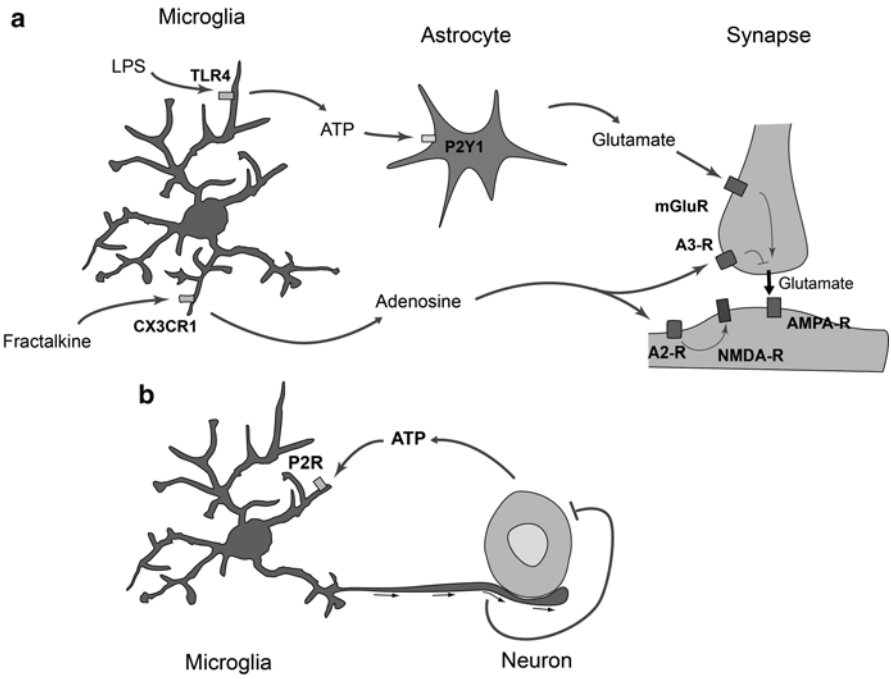

Fig. 9.4 *Microglial regulation of synapse function and plasticity: an overview of the proposed mechanisms*. (**a**) Stimulation of microglia with bacterial lipopolysaccharide (LPS) induces a rapid release of the nucleotide ATP, which binds to P2Y1 on astrocytes. Upon purinergic stimulation, astrocytes release glutamate, inducing an mGluR-dependent release of pre-synaptic glutamate. Stimulation of microglia by fractalkine induces the release of adenosine, which can decrease neuronal activity through A3R and promote NMDAR activity through A2R. (**b**) In zebrafish larva, active neurons also release ATP that attracts microglial bulbous processes. These processes decrease neuronal activity by an as yet unknown mechanism

which stimulation of microglia by fractalkine induces a significant and transient reduction of the amplitude of evoked EPSCs in CA1 pyramidal neurons (Ragozzino et al. 2006). It was further demonstrated that this reduction involves adenosine, supposedly acting on neuronal adenosine receptors 3 (A3R) (Piccinin et al. 2010). A probable mechanism of regulation is fractalkine-induced microglial release of adenosine inhibiting the pre-synaptic release of glutamate (Fig. 9.4a). Alternatively, microglia could produce ATP that is rapidly degraded into adenosine by ectonucleotidases. More recently, the same group showed that fractalkine-induced release of adenosine could also increase the hippocampal NMDA response (Scianni et al. 2013). In this study, the effects of fractalkine on the fEPSPs were abolished by a specific blocker of adenosine receptors subtype 2 (AR2), and in acute slices from AR2 knockout mice, thus proposing a role for A2R in mediating these effects. Fractalkine stimulation of microglia and astrocyte primary culture induced a modest but significant increase in the concentration of D-serine, a co-agonist of NDMA receptors. It was therefore proposed that microglial or astrocytic release of D-serine

might potentiate NMDA receptors function downstream of fractalkine signalling (Scianni et al. 2013).

A rapid regulation of neuronal activity by microglia has also been established upon stimulation of microglia by lipopolysaccharides (LPS) (Pascual et al. 2012). LPS is a specific ligand of toll-like receptor 4 (TLR4), which in the brain is exclusively expressed by microglia. Therefore, the analysis of neuronal outcomes following acute application of LPS onto brain tissue can be used to reveal relevant physiological microglia-neuron interacting pathways. Indeed, LPS stimulation of microglia in acute hippocampal slices induces a rapid and transient increase in the frequency of spontaneous synaptic AMPAergic post-synaptic currents in CA1 neurons. This effect does not occur in slices prepared from mice deficient for the myeloid-cell specific transcription factor Pu.1, which lack microglia, showing that the effect of LPS requires microglia. It was then demonstrated that upon LPS stimulation, microglia rapidly produce ATP, which recruits astrocytes (Fig. 9.4a). Astrocytes subsequently release glutamate, and this leads to increased excitatory transmission via a metabotropic glutamate receptor-dependent mechanism (Pascual et al. 2012).

The above-described studies show that stimulation of microglia modulates neuronal activity in vitro and ex vivo. The occurrence of regulating, bidirectional interactions between microglia and neuronal activity during normal physiological conditions has recently been demonstrated in vivo using confocal and two-photon imaging in the zebrafish larva (Li et al. 2012). In this system, microglia were shown to monitor spontaneous or visually evoked neuronal activity and send processes toward the active neurons, detected by their production of ATP (Fig. 9.4b). These contacts between microglial endings and active neurons further induced a rapid decrease in both frequency and amplitude of neuronal activity measured by tracking the changes in calcium events (Li et al. 2012). This study confirms and extends the data obtained upon stimulation of microglia and further demonstrates that microglia could be genuine partners of neuronal activity in the healthy brain. This role of microglia suggests that at least part of the synaptic dysfunction, observed during brain diseases, could be due to the microglial activation or dysfunction. Future work will help to elucidate the mechanisms of synaptic transmission which are specifically influenced by microglia, determine whether their structural interactions with peri-synaptic astrocytic processes are somehow involved in this regulation, and shed light on the respective roles of their interactions with pre-synaptic elements, post-synaptic elements, and peri-synaptic astrocytic processes, compared with their release of soluble mediators, in various contexts of health in the developing and mature brain in vivo.

9.5 Conclusion

Together, these combined findings from imaging, electrophysiology, and behaviour indicate that microglia in the healthy brain frequently interact with synapses, structurally and functionally, in relation with the homeostatic variations in neuronal

activity and experience. Their habitual forms of interactions include the phagocytic elimination of synaptic structures and release of soluble factors which can influence the formation, maturation, function, and plasticity of synapses, directly or indirectly. Beyond their decisive role in pathological conditions, microglia are thus emerging as important contributors to normal brain physiology. Future works will elucidate the cellular and molecular mechanisms governing their interactions with neuronal circuits, in cooperation with astrocytes and other types of glial cells, and thereby help unravel their relevance to various contexts of injury and disease.

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