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► **To cite this version:**

Maria Joana Pinto, Davide Ragozzino, Alain Bessis, Etienne Audinat. Microglial Modulation of Synaptic Maturation, Activity, and Plasticity. *Advances in neurobiology*, 2024, *Advances in Neurobiology*, 37, pp.209-219. 10.1007/978-3-031-55529-9\_12 . hal-04687728

**HAL Id: hal-04687728**

**<https://hal.science/hal-04687728v1>**

Submitted on 4 Sep 2024

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## 12. Microglial modulation of Synaptic Maturation, Activity, and Plasticity

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### **Abstract :**

Microglia, which are the resident immune cells of the CNS, also have important functions in physiological conditions. In the present chapter we review the experimental evidence that microglia modulate neuronal and synaptic activity during normal development and in adults. We show that microglia can regulate the maturation and function of both inhibitory and excitatory synapses that can be stimulated or repressed. We further review the fact that these regulations occur in various brain regions, through soluble and membrane molecules, directly or through other cell partners. This review emphasizes the fact that microglia are genuine and highly context-dependent and thus adaptable regulators of neuronal activity.

**Key words:** synapse, neuronal network, synapse maturation, neuronal development, ATP, inflammation

## 1/ Introduction

In addition to their well-known phagocytic and immunological functions, tissue resident macrophages are increasingly recognized for their role in the development and homeostasis of their host tissue, for which they acquire unique functional adaptations (Lee and Ginhoux, 2022). Microglia are no exception to the rule and over the last 20 years a growing body of evidence has shown their involvement in the development of the central nervous system (CNS), the regulation and plasticity of neurons and synapses (Tremblay et al., 2011; Mosser et al., 2017; Thion et al., 2018; Marinelli et al., 2019; Andoh and Koyama, 2021; Umpierre and Wu, 2021; Ball et al., 2022; Cornell et al., 2022; Lukens and Eyo, 2022; Mehl et al., 2022). These non-immune functions are based firstly on microglia ability to detect the activity of neurons and other glial cells. Microglia express a large repertoire of membrane receptors, transporters and ion channels through which they can sense the activity of neighboring cells (Kettenmann et al., 2011). Microglia are also endowed with a remarkable motility of their processes that permanently scan their environment (Davalos et al., 2005; Nimmerjahn et al., 2005). This motility helps detecting changes in activity and allows microglial processes to come into contact with cellular elements emitting reporting signals of activity (chemotaxis). Indeed, microglial contacts, differing in their frequency and duration, have been observed with synapses, cell bodies, axon initial segments and nodes of Ranvier and can be regulated by activity (Ikegami et al., 2019; Augusto-Oliveira et al., 2022); Chapter 8 in this book). The establishment of these contacts can be the mean by which microglia fine tune neuronal and synaptic activities by targeting specifically active, or inactive, elements. The formation of contacts between microglial processes and neurons can lead to structural modifications, such as synaptic stripping and pruning or formation of new spines (see chapter 11 in this book), but increasing evidence also indicates that microglia induce functional changes in neurons and astrocytes independently of structural modifications. Notwithstanding, Microglia do not necessarily need these contacts to release mediators, such as cytokines, growth factors and transmitters, which are also known to modulate the activity of other cells. This chapter reviews the experimental evidence indicating that microglia regulate the activity of neurons and synapses during development and in adults under physiological conditions. This field is still in its infancy

but already highlights the extreme adaptability of microglia to the heterogeneity of the functional landscape of the CNS.

## **2/ Microglia regulate synaptic maturation during development**

Microglia are derived from primitive macrophages of the yolk sac and propagate in the embryo through the blood after the establishment of the circulatory system. Immature microglia are thus present in the CNS parenchyma very early on during embryonic development (at E8.5 in the Mouse) when neurons are generated, migrate, differentiate, establish synapses and functional neuronal networks. During the last two decades, experimental evidence has shown that microglia indeed regulate several of these developmental processes (Mosser et al., 2017; Thion et al., 2018) and Chapters 9, 10 & 11 in this book). In the following paragraphs we will review how the presence of microglia influences synaptic activity during the functional maturation of neuronal networks.

### **2.1/ Loss-of-function of microglial genes alters the functional maturation of excitatory synapses**

The first evidence that microglia-neuron interactions during development regulate synaptic activity comes from studies that have analyzed excitatory synapses in mice bearing loss-of-function mutation in microglia-specific genes. DAP12 is a transmembrane protein associated with several cell-surface receptors, such as TREM2, in hematopoietic cells. Its mutation in humans leads to Nasu-Hakola disease, characterized by presenile dementia. In the CNS of mice, DAP12 is only expressed by microglia and mostly around birth (Roumier et al., 2004). It was further demonstrated that excitatory synaptic transmission at hippocampal CA3-CA1 Schaffer collateral synapses was impaired in juvenile mice deficient for DAP12 function. Electrophysiological and biochemical studies showed abnormal subunit composition of AMPA and NMDA subtypes of glutamate receptors, a decrease in synaptic TrkB receptors and enhanced long-term synaptic potentiation (Roumier et al., 2004). This indicated that loss-of-function of a receptor expressed by microglia during development has long-term consequences on the functional properties of hippocampal excitatory synapses.

The analysis of mice knockout for the fractalkine receptor CX3CR1 further confirmed that microglia do regulate the maturation of excitatory synapses. Fractalkine is a neuronal chemokine that signals to microglia through its only known receptor CX3CR1, which is exclusively expressed by microglia in the CNS (Tarozzo et al., 2002; Cardona et al., 2008; Ransohoff and Perry, 2009). In the hippocampus and in the primary somatosensory cortex, CX3CR1 deficiency leads to a

delayed colonization by microglial cells during the early postnatal period (Paolicelli et al., 2011; Hoshiko et al., 2012).

In layer 4 of this primary cortex, clusters of thalamic fibers relaying the sensory information from whiskers make excitatory synapses on the dendrites of stellate cells. These synapses are located in the center of structures named barrels whose walls are made by the soma of the stellate cells. The barrels start to form at P3-4 and the functional properties of thalamocortical synapses evolve between P5 and P12. In wild type mice microglial cells start to enter the barrel centers at P6. In *Cx3cr1* knockout mice, the overall density of microglia in layer 4 is normal but the invasion of the barrel centers is delayed by two days (Hoshiko et al., 2012). This delayed recruitment is associated with a delayed functional maturation of thalamocortical synapses: the increased incorporation of synaptic AMPA receptors, relatively to NMDA receptors (AMPA/NMDA ratio) and the switch of GluN2B to GluN2A NMDAR subunits are both impaired in developing *Cx3cr1* knockout mice. Eventually, microglia cells invade the barrel centers of *Cx3cr1* knockout mice during the second postnatal week and the functional properties of thalamocortical synapses of one-month old *Cx3cr1* knockout mice finally match those of wild type animals. Of note, the maturation of presynaptic functions, as assessed by short-term synaptic plasticity in responses to paired-pulse stimulation of thalamic fibers, was not altered in developing *Cx3cr1* knockout mice (Hoshiko et al., 2012). Impairment in the maturation of post- but not pre-synaptic components suggests that microglia control primarily the functional expression or localization of postsynaptic receptors at developing thalamocortical synapses.

In the hippocampus of *Cx3cr1* knockout mice the recruitment of microglia is also delayed between the second and fourth postnatal weeks and this is also associated with an impaired maturation of excitatory synapses of CA1 pyramidal neurons (Paolicelli et al., 2011). During normal development the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) becomes larger than that of miniature EPSCs (mEPSCs). This increase in the sEPSC/mEPSC amplitude ratio, also referred as synaptic multiplicity, reflects increased connectivity and redundancy of afferent synaptic inputs during development. This increase in synaptic multiplicity did not occur in P13-P16 *Cx3cr1* knockout mice. In addition, the frequency and the amplitude of mEPSCs were enhanced in *Cx3cr1* knockout mice. It was thus proposed that *Cx3cr1* knockout mice show an excess of weak excitatory synapses as a result of a failure of microglia to eliminate immature connections (Paolicelli et al., 2011), even though the same group did not detect evidence of synapse phagocytosis in a further study (Weinhard et al., 2018). Subsequent studies reported that several synaptic dysfunctions persist in adult *Cx3cr1* knockout mice (Zhan et al., 2014; Basilico et al., 2019). These observations show that hippocampal excitatory synapses of

*Cx3cr1* knockout mice have an abnormal development and remain functionally impaired in adulthood. In addition, they also stress that deficiency in fractalkine signaling can lead to different functional impairments of developing excitatory synapses in two different brain areas (hippocampus versus somatosensory cortex). Noteworthy, no alteration of the activity-dependent developmental plasticity could be detected in the visual cortex of CX3CR1 deficient mice (Lowery et al., 2017; Schechter et al., 2017). Therefore, fractalkine signaling, and probably microglia in general, is not a universal modulator of synaptic maturation and plasticity. Specific patterns of development in different brain areas may determine specific microglia phenotypes that in turn have different and specific influence on synaptic functional maturation.

Recently, analysis of knockout mice for the two-pore domain halothane-inhibited K<sup>+</sup> (THIK-1) channel has also provided evidence for the involvement of microglia in regulating excitatory synaptic transmission during the development of the hippocampus (Izquierdo et al., 2021). THIK-1 is the main K<sup>+</sup> channel expressed by microglia and a previous study showed that THIK-1 channels maintain ramification of microglial processes and their surveillance of the brain parenchyma (Madry et al., 2018). Recordings of CA1 pyramidal neurons in acute hippocampal slices of P17 to P19 mice showed that the frequency, but not the amplitude, of sEPSCs and mEPSCs was twice larger in THIK-1 knockout animals compared to wildtype mice. This increased frequency in EPSCs was interpreted as the result of a deficit of microglia in pruning excitatory synapses during development, suggested by a lower phagocytic activity of microglia and a higher number of hippocampal excitatory synapses in THIK-1 knockout mice (Izquierdo et al., 2021). Interestingly, EPSC frequency and the number of excitatory synapses did not differ between adult knockout and wildtype mice, suggesting that another mechanism can correct, on the long term, these developmental synaptic dysfunctions.

Noteworthy, the studies on constitutive DAP12, CX3CR1 and THIK-1 knockout mice have revealed a contribution of microglia to the regulation of synaptic activity during development. Yet, the actual mechanisms by which these microglial alterations affect synaptic transmission remain poorly identified.

## **2.2/ Microglia also regulate the maturation of inhibitory synapses**

The studies on the influence of microglia on the development of synaptic networks have been mostly focused on excitatory synapses. Yet, recent evidence indicates that microglia also contribute to the functional maturation of inhibitory synapses. Transient depletion of microglial cells during embryonic development, which affects the laminar positioning of subsets of neocortical interneurons (Squarzoni et al., 2014), leads to a two-fold increase in the amplitude

of unitary synaptic currents mediated by parvalbumin (PV) fast-spiking interneurons onto excitatory neurons of layer 4 of the somatosensory cortex of the offspring at P20 (Thion et al., 2019). In addition, axonal arborization of PV interneurons which is usually confined to their home-barrel (Koelbl et al., 2015) extended over several adjacent barrels after embryonic microglia depletion. In vivo recordings in anesthetized mice showed that this hyper-inhibition led to a significant slowdown of the lateral propagation of sensory information in the somatosensory cortex. This hyper-inhibition was transient during postnatal development and synaptic inhibition mediated by PV interneurons was, on the contrary, lower in the cortex of P60 mice after embryonic microglial depletion compared to control animals (Thion et al., 2019). The mechanisms responsible for this biphasic effect of microglia depletion on the developmental trajectory of cortical inhibition remain unknown. Interestingly, however, a similar biphasic impact on cortical inhibition (i.e. hyper- followed by hypo-inhibition) was also observed in the offspring after maternal immune activation (MIA) induced at E13.5 via intraperitoneal injections of lipopolysaccharide (LPS), a mouse model of neurodevelopmental disorders (Patterson, 2011). Of note, the similarity of the features and of the temporality of the phenotypes observed after MIA or prenatal microglia depletion suggests that the defects induced by MIA on the development of cortical inhibition result from an inability of microglia to fulfill their normal physiological roles, rather than from an aberrant or excessive immune response.

Depleting microglia during early postnatal development (P1-P15) with an antagonist (PLX5622) of the colony stimulating factor 1 receptor (CSF1R) also impairs the development of inhibitory synapses in the mouse somatosensory cortex (Favuzzi et al., 2021). At P15, the number of synapses made by PV (but also by somatostatin) interneurons onto excitatory neurons was increased in depleted mice compared to control animals. This was associated with an increased amplitude of the synaptic responses induced in excitatory neurons by optogenetic activation of PV interneurons. The frequency, but not the amplitude, of miniature inhibitory postsynaptic currents (mIPSCs) was also increased in depleted mice. This action of microglia on inhibitory synapses was mediated by specialized GABA-receptive microglia and was limited to a critical period of postnatal development: stopping the PLX6622 treatment at P12, instead of P15, or treating the mice between P15 and P30 failed to modify the number of PV synapses in the somatosensory cortex. In the primary visual cortex, whose functional maturation is delayed compared to the somatosensory cortex, PLX5622 treatment needed to be extended up to P25 (P1-P25) to increase the number of PV synapses. Finally, STED microscopy analysis of contacts between PV synapses and microglia and electrophysiological recordings in C1q knockout mice suggest that microglia control inhibitory synaptic transmission during postnatal development

through complement-dependent synaptic pruning mechanisms (Favuzzi et al., 2021). Thus, microglia regulate the maturation of inhibitory synapses during critical periods of postnatal development and dysfunction of microglia impacts on the E/I balance in the developing cortex.

In the cerebellum a co-regulation of excitatory and inhibitory synapse development by microglia has also been observed. Purkinje cells are innervated by only one climbing fiber that makes a powerful dendritic glutamatergic synapse. At birth, several climbing fibers impinge onto Purkinje cells and after P7 one climbing fiber is selectively strengthened while others are progressively eliminated (Lohof et al., 1996). Microglia depletion impairs this developmental elimination of climbing fibers and this effect appears to be independent of a deficit in synaptic. Instead, the impact of microglia depletion was shown to be the consequence of an impaired maturation of GABAergic synapses onto Purkinje cells, which is known to be required for the regression of multiple innervation (Nakayama et al., 2012). Indeed, microglia-specific *Csf1r* knockout mice in which the density of microglia was severely reduced (> 70%), display a lower number of GABAergic synapses and a decrease in the amplitude and the frequency of mIPSCs. Remarkably, promoting inhibitory transmission by treating *Csf1r* knockout mice with the positive modulator of GABAergic synapses diazepam, restored climbing fiber elimination (Nakayama et al., 2018). This study nicely illustrates the homeostatic changes that result from an initial perturbation of microglia: microglia are crucial for the formation of functional inhibitory synapses and perturbing this maturation impacts on the elimination of supernumerary climbing fibers.

Microglia therefore play an important role in the functional maturation of synapses during the development of several brain areas. Fractalkine and GABA are two signaling pathways that allow developing neurons to mobilize microglia. However, the actual mechanisms by which microglia modulate the functional maturation of synapses remain poorly understood.

### **3/ Modulation of synaptic function and plasticity by microglia**

In the physiological adult brain, microglia rapidly react to neuronal activity by modulating the frequency or the duration of the contacts that they establish with neuronal elements, including synapses (Wake et al., 2009; Tremblay et al., 2010; Li et al., 2012; Pfeiffer et al., 2016). Microglia also react to stimulation by producing a large repertoire of signaling factors that are known to control synaptic function, such as cytokines, neurotransmitters or extracellular matrix proteins. Microglia are thus putative sensors and regulators of neuronal activity. Recent evidence in support of such a role has arisen from *in vivo* studies performing depletion of microglia in adult animals, thus avoiding the confounding impact of perturbing microglia during development. Microglia depletion had initially been accomplished by expression of diphtheria toxin receptors



specifically on microglia (Parkhurst et al., 2013). It is now mostly done via inhibition of CSF1R, which is required for microglia survival (Elmore et al., 2014). Conveniently, these strategies do not affect animals' health and do not cause gross changes in the brain tissue in which neuronal density and the general organization of the network are maintained (Parkhurst et al., 2013; Elmore et al., 2014; Wang et al., 2016). Depletion is thus becoming a common tool used in many studies to assess microglia roles, in particular in the regulation of synaptic function and plasticity.

In the adult hippocampus, depletion experiments have revealed that microglia exert a versatile context-dependent regulation of synaptic activity. For instance, in the absence of microglia, basal glutamatergic synaptic transmission in the hippocampus is weakened (Yegla et al., 2021; Basilico et al., 2022; Corsi et al., 2022). Indeed, patch-clamp recordings from CA1 pyramidal neurons reveal a pronounced decrease in the amplitude of spontaneous and evoked EPSC but no change in the amplitude of mini EPSC, suggesting that microglia depletion has not impacted the functionality of postsynaptic receptors (Yegla et al., 2021; Basilico et al., 2022). Accordingly, microglia-depleted hippocampal slices displayed an increased paired-pulse ratio (Basilico et al., 2022; Corsi et al., 2022), suggestive of a defect of presynaptic release, and reduced synaptic multiplicity (Basilico et al., 2022), a measure of the density of functional neurotransmitter release sites (Sunstrum and Inoue, 2019). However, the amplitude of long-term potentiation (LTP), a model of activity-induced synaptic plasticity, is increased at CA1 synapses in microglia-depleted hippocampus (Basilico et al., 2022; Corsi et al., 2022). The results of these depletion experiments thus suggest that under physiological condition microglia strengthen basal glutamatergic transmission but inhibit activity-dependent potentiation of synaptic strength.

Microglia depletion studies recently added another aspect of microglia as regulator of synaptic function during sleep. Excitatory synaptic transmission is downscaled during sleep to offset the saturation of synaptic strength occurring during wake (Liu et al., 2010; Tononi and Cirelli, 2014). Accordingly, during sleep, hippocampal CA1 pyramidal neurons show decreased amplitude of mEPSCs, as well as decreased amplitude and frequency of sEPSCs (Corsi et al., 2022). Strikingly, microglia depletion abolishes sleep/wake differences in synaptic strength (Corsi et al., 2022), establishing microglia as physiological regulators of functional synaptic plasticity.

These depletion experiments in the hippocampus show that microglia can regulate many aspects of synaptic function, depending on the context. This versatile context-dependent regulation of synaptic activity is even more striking in the cortex. In the motor cortex, depletion of microglia decreases the frequency of mEPSCs recorded in pyramidal cells (Parkhurst et al., 2013). In the visual cortex, microglia depletion increases excitatory and inhibitory drive onto pyramidal

neurons in acute slices and this was associated with enhanced activity of both excitatory and inhibitory neurons in vivo (Liu et al., 2021). Enhanced frequency of mEPSCs, with no change in mIPSCs, in pyramidal cells of the visual cortex was also observed upon microglia depletion of younger mice (P28-30) (Ma et al., 2020). In this latter example, changes in synaptic functions upon microglia depletion were associated with the inhibition of ocular dominance plasticity. This plasticity is elicited by occluding visual inputs to one eye during late postnatal development, which leads to robust shifts in the responsiveness of cortical neurons towards the non-occluded eye (Hofer et al., 2006; Hooks and Chen, 2020). Ocular dominance shifts involve reorganization of the local circuitry including structural remodeling of synapses and long-term synaptic plasticity. Microglia depletion prevents the shift in ocular dominance triggered by closure of one eye, as assessed by measuring the ratio between the neuronal responses evoked by a visual stimulus to each eye (Ma et al., 2020). Attempts to characterize the molecular microglial machinery involved ruled out the fractalkine receptor CX3CR1 (Lowery et al., 2017; Schechter et al., 2017) and led to the identification of the purinergic receptor P2RY12 (Sipe et al., 2016). Closure of one eye alters microglia interactions with the synaptic cleft and increases microglial inclusions of synaptic proteins in a P2RY12-dependent manner (Sipe et al., 2016). These latter observations, together with the reported increase in mEPSC frequency during microglia depletion (Ma et al., 2020) suggest that regulation of ocular dominance plasticity by microglia occurs through the modulation of excitatory synapses in the visual cortex.

These different effects of microglia ablation may reflect regional heterogeneity in microglial regulation of synaptic function. Alternatively, and not exclusively, it may also reflect indirect effect of different depletion strategies. For instance, different durations of depletion may lead to different effects on the formation and elimination of synapses (Parkhurst et al., 2013; Ma et al., 2020). Finally, it cannot be excluded that at least some of the effects observed upon depletion of microglia are secondary to other long-term alterations. For instance, some studies have reported mild astrocytic reactions (Wang et al., 2016; Yegla et al., 2021; Basilico et al., 2022; Du et al., 2022), which may impair the astrocytic regulation of synaptic functions. Moreover, effects on peripheral immune cells following CSF1 receptor inhibition have been recently described (Lei et al., 2020), thus questioning the specific action of this approach at the CNS level. Also, microglia depletion may have led to compensatory changes that can indirectly affect synaptic transmission. Such compensatory mechanisms have been described in the retina. In this structure, microglia depletion leads to degeneration of the presynaptic terminals of photoreceptors, which likely underlies the reduction in the amplitude of light-evoked electroretinographic responses (Wang et al., 2016). Nevertheless, no deleterious effects were

observed on visual acuity suggesting compensatory mechanisms maintaining the integrity of retinal functions despite alteration of the synaptic network. Adopting acute and/or more localized strategies to interfere with microglia in future work may avoid secondary effects to understand better the actual role of microglia in synaptic function in discrete networks (Badimon et al., 2020).

These studies using depletion of microglia demonstrated that microglia contribute to the regulation of synaptic functions but they have not allowed the identification of the molecular mechanisms of this regulation.

#### **4/ Microglial signaling pathways controlling synaptic functions**

##### **4.1/ TLR4 Signaling function**

Lipo-polysaccharide (LPS) is an essential component of the bacterial wall of Gram-negative bacteria that can trigger CNS inflammation. LPS binds to Toll-Like Receptor 4 (TLR4), which is specifically expressed by microglia in the CNS (Pascual et al., 2012). Application of LPS has been therefore used to reveal endogenous mechanisms of microglia-to-neuron communication. This strategy was used as a proof of concept to demonstrate the ability of microglia to regulate synaptic functions and to identify molecular actors of these regulations. For instance, in hippocampal slices acute application of LPS induces microglial release of ATP that recruits astrocytes via P2RY1 receptors. The activation of this signaling pathway induces the release of glutamate by astrocytes, leading to a mGluR-mediated increase in the frequency of mEPSCs and favoring the generation of epileptiform activities (Pascual et al., 2012). In the spinal cord, the same strategy was used to assess how microglia could regulate inhibitory synapses. Interestingly, stimulation of microglia selectively modulates the strength of glycinergic but not GABAergic synapses (Cantaut-Belarif et al., 2017). This effect was due to the release of prostaglandin E2 (PGE2) that changes the diffusion dynamic of GlyR, but not GABAAR, leading to a reduction in the accumulation of GlyRs at synapses and of the amplitude of glycinergic IPSCs.

In rat cerebellar slices, acute activation of microglial TLR4 pathway by LPS increases intrinsic excitability of Purkinje neurons. This effect is independent of ATP signaling but relies on microglial tumor necrosis factor alpha (TNF $\alpha$ ) (Yamamoto et al., 2019). In addition, LPS also enhances excitatory synaptic transmission (mEPSC frequency and amplitude). This latter effect is blocked by a purinergic receptor antagonist and could be mimicked by ATP application, suggesting the involvement of purinergic signaling in the LPS-induced modulation of cerebellar

excitatory synapses. Interestingly, local infusion of LPS or TNF $\alpha$ , but not ATP, in anterior lobes of the cerebellar vermis of living animals induced depressive-like behavior that was abolished when inhibiting TNF $\alpha$  signaling or depleting microglia (Yamamoto et al., 2019).

Altogether these studies show that activation of microglial TLR4 induces rapid (within few minutes) changes in synaptic activities and neuron excitability in different CNS areas that probably contribute to behavioral modifications, such as depression and sick behavior, associated with inflammatory conditions. They also illustrate the different signaling pathways (purinergic, release of PGE2 or TNF $\alpha$ ) by which microglia can impact onto synaptic and neuronal functions, independently of synapse formation and elimination.

#### **4.2/ TNF $\alpha$ signaling**

Amongst microglial molecules with a putative role in synaptic regulation, TNF $\alpha$  deserves a special attention. Actually, TNF $\alpha$  has long been identified as a cardinal modulator of several aspects of synaptic function. TNF $\alpha$  controls basal excitatory synaptic transmission as well as long-term potentiation in hippocampal slices (Tancredi et al. 1992). TNF $\alpha$  was also demonstrated to regulate both excitatory and inhibitory synapses (Beattie et al., 2002; Pribiag and Stellwagen, 2013) and to contribute to the synaptic modulation occurring in pathologies (Berta et al., 2014; Lewitus et al., 2016). Finally, TNF $\alpha$  was shown to control basal synaptic strength by supporting glutamatergic gliotransmission (Santello et al., 2011) and homeostatic adjustment of synaptic strength during synaptic scaling by regulating synaptic trafficking of AMPARs (Beattie et al., 2002; Stellwagen and Malenka, 2006). However, the identification of cells producing TNF $\alpha$  in these conditions has remained elusive. Transcriptomic analysis shows a near exclusive expression of TNF $\alpha$  by microglia in the brain (Zamanian et al., 2012; Zeisel et al., 2018), thus suggesting a prominent role of microglia in TNF $\alpha$ -mediated control of synapses. This hypothesis has been confirmed in the nucleus accumbens where microglia release of TNF $\alpha$  reduces AMPA/NMDA ratio on D1-expressing medium spiny neurons and antagonizes cocaine-induced synaptic plasticity (Lewitus et al., 2016). Moreover, in the upper layers of the cortex, microglia release of TNF $\alpha$  controls an activity-dependent plasticity of inhibitory synapses by promoting the synaptic enrichment of GABA<sub>A</sub> receptors. Interestingly, this plasticity likely underlies sleep-dependent memory consolidation, which is blunted in microglia TNF $\alpha$  knockout mice (Pinto et al., 2022); see below, paragraph 5). Of note, peripheral TNF $\alpha$  can also cross the blood brain barrier and change synaptic properties. TNF $\alpha$  produced upon peripheral inflammation modulates the properties of hippocampal glutamatergic EPSCs (Riazi et al., 2008, 2015) and spine turnover in

the cortex (Garré et al., 2017). These studies highlight a role of TNF $\alpha$  as an interface between systemic inflammation and functional synaptic changes in the brain.

#### **4.3/ Fractalkine signaling**

Fractalkine, also known as CX3CL1, is a chemokine with a major role in neuron-to-microglia communication. Fractalkine is produced by neurons and its only known receptor, CX3CR1, is exclusively expressed by microglial cells in the CNS (Cardona et al., 2006). The modulatory role of fractalkine was initially demonstrated by its application onto neuronal culture that induced a strong and rapid modulation of calcium currents in neurons (Meucci et al., 1998) as well as a temporary depression of AMPA receptor-mediated currents (Limatola et al., 2005). Further studies showed that fractalkine exerted similar effect in slices of hippocampus, depressing glutamatergic synaptic currents and field potentials (Bertollini et al., 2006; Ragozzino et al., 2006) and impairing LTP (Maggi et al., 2009). Fractalkine application was also observed to cause the production of adenosine by microglia and adenosine receptors are responsible for fractalkine-induced synaptic modulation. Indeed, both fractalkine-induced depression of AMPA-mediated currents (Piccinin et al., 2010) and LTP impairment are AR3-dependent (Maggi et al., 2009). Fractalkine treatment also causes A2AR-dependent release of D-serine from microglia or surrounding astrocytes, which then potentiated NMDAR transmission (Scianni et al., 2013). Besides these acute effects of fractalkine on excitatory transmission, Rogers and collaborators showed that hippocampal long-term potentiation was impaired in CX3CR1 knockout mice (see however Maggi et al., 2009; Maggi et al., 2011). This LTP impairment was shown to be due to an overproduction of IL1 $\beta$  in CX3CR1 knockout mice (Rogers et al., 2011).

#### **4.4/ Extracellular vesicles**

Extracellular vesicles (EVs) are small vesicles that are released by cells and that can transfer lipids, proteins and RNAs from one cell to another. Microglia are known to shed EVs after stimulation of P2RX7 receptors by ATP (Bianco et al., 2005) and it has been proposed that these EVs can modulate synaptic activity. Actually, EVs harvested from ATP-stimulated cultured microglia could potentiate synaptic glutamate release, increase the frequency of EPSC and release probability at glutamatergic synapses in hippocampal culture (Antonucci et al., 2012). Of note, EVs derived from inflammatory microglia regulated synaptic activity in an opposite direction, reducing mEPSCs frequency in cultured hippocampal neurons, supposedly through specific miRNA (Prada et al., 2018). It has also been recently proposed that TRPV1 stimulation promotes microglia EVs shedding that increases mEPSCs frequency in mouse cortical slices (Marrone et al., 2017). The functional relevance of these mechanisms remains to be firmly established but it emphasizes

the putative roles of membrane contact-based signaling in the regulation of neuronal function by microglia.

Altogether, these studies revealed the role of microglia in controlling the activity of synapses and neurons. Accordingly, a growing number of studies highlight microglia as part of integrated regulatory networks ensuring homeostatic regulation of synaptic activity

### **5/ Reciprocal microglia-neuron interactions regulating neuronal networks**

A pioneering study linking microglia and synaptic function was carried out in the spinal cord in the context of neuropathic pain (Coull et al., 2005). This work reported that ATP released in the dorsal horn of the spinal cord upon peripheral nerve injury, triggers the release of microglial brain-derived neurotrophic factor (BDNF). BDNF induces downregulation of neuronal KCC2 chloride transporter, thereby increasing intracellular chloride and inverting the polarity of GABA<sub>A</sub> receptor (GABA<sub>A</sub>R)-mediated currents, ultimately leading to higher neuronal excitability and nociceptive hypersensitivity (Ferrini et al., 2013). Further studies demonstrated the involvement of calcium permeable P2X4 receptors and their upregulation in spinal cord microglia in response to peripheral nerve injury (Ulmann et al., 2008). Strikingly, microglia involvement in this model of neuropathic pain seems to occur only in males (Sorge et al., 2015; Mapplebeck et al., 2016).

The possible involvement of microglia in the regulation of neuronal networks has also been investigated in physiological contexts and several examples showed that microglia dampen excessive neuronal activity. This homeostatic role relies on an efficient neuron-to-microglia communication in which ATP has a central role. Indeed, ATP is released by neurons during intense neuronal activity and triggers the reaction of microglia that express a large repertoire of P2 receptors (Hickman et al., 2013; Dissing-Olesen et al., 2014; Eyo et al., 2014). For instance, a feedback inhibitory effect of microglia on neuronal activity has been shown in the zebrafish larvae (Li et al., 2012). In the tectum, microglial processes are attracted by the most active neurons detected by their production of ATP. The formation of these contacts between microglial endings and the soma of active neurons induce a rapid decrease in both frequency and amplitude of spontaneous and light-induced neuronal activity assessed by Ca<sup>2+</sup> imaging (Li et al., 2012).

A consistent rapid negative regulation of neuronal activity has also been recently documented in the mouse somatosensory cortex (Merlini et al., 2021). In this structure, neurons activated by whiskers stimulation are preferentially contacted by microglia. However, this preferential contact was lost in mice with genetic inhibition of microglial Gi-protein signaling and, in these animals, neuronal stimulation is associated with spontaneous seizures and hypersynchronization of

neuronal activity. These experiments further suggest that a dynamic microglia-neuron interaction prevents neuronal hyperexcitability.

Recently, the mechanism by which microglia inhibit neuronal activity has also been uncovered in the striatum (Badimon et al., 2020). This study showed that in different models of pharmacologically induced seizures, ATP released upon high neuronal activity attracts microglial processes and is subsequently converted into AMP by the microglial ectonucleotidase CD39 and then into adenosine by another ectonucleotidase CD73 expressed by microglia and other cell types. In turn, adenosine mediates the suppression of neurotransmission in D1 neurons via activation of A1R. Accordingly, depletion of microglia specifically in the striatum was shown to cause synchronization of neuronal activity and increased seizure susceptibility (Badimon et al., 2020). Thus, this study shows that purinergic signaling is responsible for the reciprocal neuron-microglia interaction: Neuronal ATP and P2RY12 receptors mediate the attraction of microglial processes to active neurons where microglial ectonucleotidases can promote the production of adenosine that inhibits neurons.

On the contrary, the work Akiyoshi et al (2018) in the cortex of awake mice proposed that the establishment of direct contact between a microglial process and a single active synapse results in a specific increase in the activity of the contacted synapse, as seen with  $Ca^{2+}$  imaging of single spines. This increase in synapse activity induced by microglia contact was not observed when microglia were reactive in mice injected with LPS. Furthermore, microglia depletion or LPS injection decreased synchronization of neuronal activity in the cortex (Akiyoshi et al., 2018), suggesting that microglia-induced increase in synaptic activity may contribute to the synchronization of local populations of cortical neurons. The opposite effect of microglia depletion on neuronal synchronization in the striatum (Badimon et al., 2020) and in the cortex (Akiyoshi et al., 2018) suggests that microglial impact in the neuronal network is region-specific, likely to rely on a different neuron-microglia crosstalk. Alternatively, these differences may also be due to the level of neuronal activity that recruits microglia. The same group that showed an increased activity of cortical synapses contacted by microglia (Akiyoshi et al., 2018) previously reported that strong neuronal activity in cortical slices that leads to axonal swelling and sustained depolarization of soma membrane potential induces the recruitment of microglial processes to the swollen axons and a rapid soma repolarization (Kato et al., 2016).

Finally, microglia were also shown to control the activity-driven regulation of inhibitory synapses during sleep. In the upper layer of the cortex, NMDA stimulation of pyramidal cells leads to upregulation of synaptic GABA<sub>A</sub>Rs specifically at somatostatin interneurons inputs onto

pyramidal neurons, known as inhibitory long-term potentiation (iLTP) (Chiu et al., 2018). Recently, Pinto et al. demonstrated that induction of iLTP requires microglial TNF $\alpha$ . It was proposed that microglia sense neuronal activity through an ATP/P2RX7 signaling pathway and respond to it by releasing TNF $\alpha$  (Pinto et al., 2022). Microglial TNF $\alpha$  then gates the phosphorylation of neuronal CaMKII that modulates synaptic GABA $_A$ R content. Strikingly, this pathway is likely recruited during sleep since modulation of synaptic GABA $_A$ R that normally occurs during sleep wake cycle was not observed in mice with specific deletion of microglial TNF $\alpha$  or in P2RX7 knock out mice (Pinto et al., 2022).

## **6/ Reciprocal Microglia-neuron interactions regulate structural plasticity of synapses.**

Besides supporting plastic adjustments to the strength of synapses, microglia are also involved in structural plasticity of synapses in adulthood. Experience-dependent spine turnover is believed to alter circuit connectivity that supports novel learning and memory traces (Caroni et al., 2012). In the motor cortex, spines are permanently formed and eliminated and the rate of formation/elimination increases when mice engage in motor-skill learning like running on an accelerating rotating rotarod (Parkhurst et al., 2013). Both basal and motor learning-related spine formation and elimination were decreased by short-term microglia depletion, and spine formation, but not elimination, was decreased by microglia-specific deletion of BDNF (Parkhurst et al., 2013). Moreover, both manipulations result in slower performance improvement on the rotarod indicating impaired motor learning (Parkhurst et al., 2013). This pioneer study provided the first proof that microglia can shape circuits by controlling structural synaptic plasticity upon learning. How this function is achieved by microglia-secreted BDNF has remained unexplored. A compelling mechanism underlying microglia control of experience-dependent synapse remodeling has been proposed in the hippocampus (Nguyen et al., 2020). Experience primes neurons for synaptic plasticity by controlling their expression of the cytokine IL-33. High neuronal secretion of IL-33 activates the IL-33 receptor on nearby microglia which in turn promotes clearance of extracellular matrix proteins and subsequently allows formation of new spines (Nguyen et al., 2020). In support of microglial remodeling of synapses through changes in the extracellular environment, electron microscopy of microglia-labeled tissue reveals larger extracellular space around microglia processes (Tremblay et al., 2010).

Microglia have long been proposed as actors in the displacement of synaptic terminals following nerve lesions known as synaptic stripping (Blinzinger and Kreutzberg, 1968).& More recently, Chen and colleagues proposed that after repeated LPS injections, reactive microglia modulate



inhibitory synaptic transmission by displacing presynaptic terminals from neurons in the mouse cortex (Chen et al., 2014). This phenomenon is associated with the reduction of GABAergic transmission and increase of neuronal firing synchronization (Chen et al., 2014). While this mechanism is difficult to associate with functional synaptic regulation, its importance during infantile complex febrile seizures has recently been proposed (Wan et al., 2020). This study proposed that microglia contact with neuronal somata abolished the increase of GABAergic transmission and neuronal excitability induced by complex febrile seizures. Microglia would reduce susceptibility to seizures through the displacement of GABAergic synapses from cortical neuron somata. This protective effect of microglia is P2RY12-dependent, once more implicating neuron-to-microglia ATP signaling, and might represent a mechanism for the fine tuning of brain microcircuits through the control of inhibition (Wan et al., 2020).

## **7/ Conclusions**

Regulation of synapses by microglia has been demonstrated for both excitatory and inhibitory synapses that can be stimulated or repressed at basal level or upon activity. Such regulations occur in several regions of the nervous system and concern long-term and short-term regulations of both the pre- and the post-synaptic compartments. In addition, these regulations are performed through a variety of molecules that are soluble or membranous and can be direct or involve a third cellular partner. Microglia are thus genuine and extremely versatile regulators of synapse functions. A possible explanation of such versatility could be that, in agreement with their immune origin, microglia are actually extremely sensitive to their environment and able to adapt their mode of regulation to their partners and the cellular context.

Despite the extreme diversity of microglial regulatory processes, two points deserve more attention. First, the neuron to microglia communication often occurs via adenosine and phosphorylated adenosine molecules (ATP, ADP, AMP). Indeed, microglia are highly reactive to purinergic stimuli as they express a wide array of P2 receptors. Accumulating evidence suggests that microglia use these molecules as a proxy of neuronal activity and the health status of neurons. Second, membrane - membrane communication seems to be a major actor of microglia-neuron communication. For instance, CX3CR1/Fralktine are both membrane protein; TNF $\alpha$  exist in both soluble and membrane form; PGE2 is a lipid and thus membrane associated, IL1b can also be produced through EVs which are themselves a major communication vector. Despite the large number of studies on the formation of contacts between microglia and

different neuronal compartments, possible involvement of membrane-membrane interactions in the regulation of neuronal activity by microglia may have been overlooked.

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