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The neuroimmunological synapse: from synaptic homeostasis to brain disease

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Abstract: Microglia are the resident immune cells of the central nervous system (CNS). They play fundamental roles in active immune defense and neuroinflammatory responses. Historically, it has been assumed that microglia exist in a resting state until pathological stimuli trigger their activation. However, a series of recent landmark studies revealed important physiological functions of microglia in neural development, synaptic remodeling and homeostasis. Likewise, accumulating evidence suggests that immune mediators and inflammatory cytokines may assert physiological roles in synaptic transmission and plasticity. Hence, the concept of a *neuroimmunological synapse* has started to emerge based on the observation that microglial factors, such as tumor necrosis factor alpha (TNFα) modulate plasticity at tripartite synapses. In pathological conditions, in which microglia are activated by non-physiological stimuli (and/or circulating immune mediators and immune cells enter the CNS), homeostasis between microglia, astrocytes and neurons at synaptic sites will be altered, which may initiate, promote or sustain pathological brain states.

Keywords: Neuroinflammation, Synaptic Plasticity, Plasticity, TNFα, Synaptopodin, Microglia

Zusammenfassung: Mikroglia sind Zellen des angeborenen Immunsystems im zentralen Nervensystem, die eine bedeutende Rolle bei entzündlichen Veränderungen im Nervengewebe spielen. Ursprünglich galt die Annahme, dass Mikrogliazellen ihre Funktion erst nach Aktivierung durch pathologische Stimuli aufnehmen. Neuere Studien deuten darauf hin, dass Mikroglia physiologische Funktionen bei neuronalen Entwicklungsprozessen oder synaptischen Anpassungsreaktionen hat. Basierend auf dem Konzept, dass die Freisetzung mikroglialer Faktoren synaptische Eigenschaften tripartiter Synapsen (Präsynapse, Postsynapse, Astrozyt) beeinflussen kann, wurde der Begriff der *neuroimmunologischen Synapse* geprägt. Unter Bedingungen, bei denen der Aktivitätszustand der Mikroglia durch endogene oder exogene pathologische Stimuli verändert wird, kann dadurch das physiologische Zusammenspiel von Mikroglia, Astrozyten und Nervenzellen an Synapsen gestört sein, wodurch krankhafte Prozesse im zentralen Nervensystem angestoßen, befördert oder erhalten werden können.

Schlüsselwörter: Neuroinflammation, Synaptische Plastizität, TNFα, Synaptopodin, Mikroglia

Introduction

The characterization of structure-function interrelations in the central nervous system (CNS) was significantly advanced in the end of the 19th century when Franz Nissl developed a new staining method which allowed for the visualization of the CNS cytoarchitecture. Early neuropathological investigations pointed to a non-neuronal, i. e., glial cell type (Virchow, 1846), which showed intriguing similarities to macrophages of the immune system. These intricate glial cells were further characterized based on modified Golgi staining protocols (Robertson, 1899) which revealed their ramified appearance and the comparatively small cell bodies (c.f., Fig. 1*A*). Eventually, Pío del Río Hortega named this class of glial cells 'microglia' (Cajal, 1920).

In the 1960 s, the first transmission electron microscopy images of microglia were published [(Schultz et al., 1957) c.f., Fig. 1*B*]. These ultrastructural studies provided direct experimental evidence for the earlier proposed phagocytic properties of microglia (Penfield, 1925). It was soon suggested that microglia could be involved in the removal of dysfunctional neuronal synapses [(Gray, 1959); c.f., Fig. 1*C* and Table 1], a phenomenon termed 'synaptic stripping' (Blinzinger and Kreutzberg, 1968; Kettenmann et al., 2013). Several years later, the role of microglia as resident immune cells of the CNS was firmly established (Giulian and Baker, 1986), also pointing towards the relevance of dynamic properties of microglia – long before *in*

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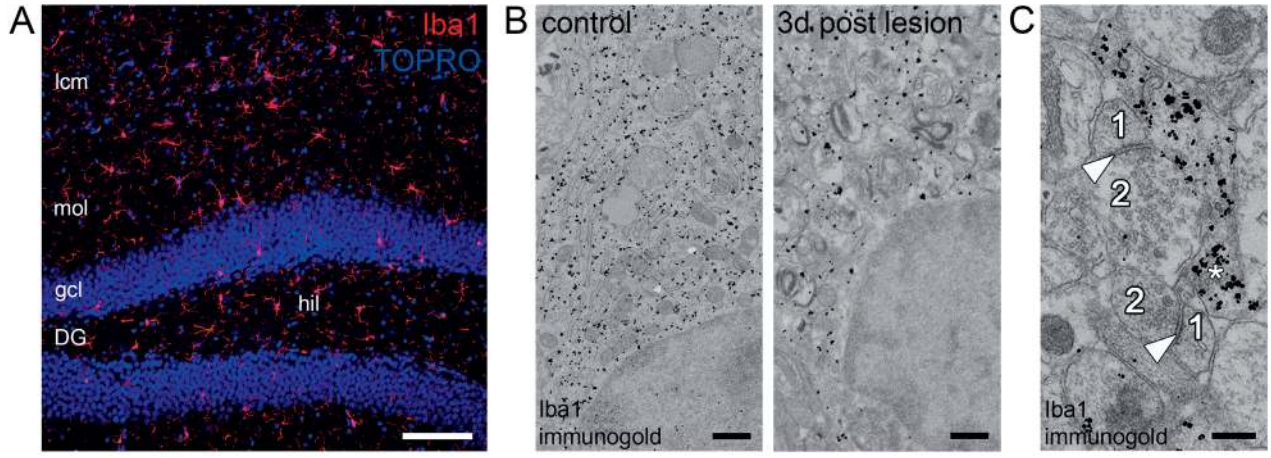

Figure 1: Microglia are small ramified cells that interact with synapses (A) Iba1 immunostaining of the mouse dorsal hippocampus reveals the morphology and distribution of microglia under physiological conditions. TOPRO nuclear staining was used to visualize cyto-architecture. (DG, dentate gyrus; gcl; granule cell layer; mol, stratum moleculare; lcm, stratum lacunosum; hil, hilar region). Scale bar, 100 µm. (B) Transmission electron micrograph of Iba1 immunogold labeled microglia in the molecular layer of a three-week-old hippocampal tissue culture. Numerous inclusion bodies are detected 3 days after lesioning the entorhino-hippocampal fiber tract *in vitro*. Scale bars, 500 nm. (C) Microglial processes (asterisk) in close proximity to neuronal synapses. Postsynaptic compartments indicated by '1', arrow heads point to synaptic clefts, and '2' indicates presynaptic compartments. Scale bar, 250 nm.

Table 1: Cell-to-cell contact sites

Name	Description	Reference (example)
Neuronal (electrochemical) Synapses	Presynaptic specialization + synaptic cleft + postsynaptic specialization (c.f., Fig 1C)	(Gray, 1959)
Tripartite Synapses	Neuronal Synapses + astrocytic endfeet	(Panatier et al., 2014)
Quadpartite Neuroimmunological Synapses	Tripartite Synapse + Microglia	(Schafer et al., 2013)
Immune Synapses	Leukocyte/Leukocyte-Interactions	(Llodra, 2017)
Enteroendocrine-Vagal-Synapses	Enteroendocrine/Vagal Nerve-Interactions	(Kaelberer et al., 2018)

vivo multiphoton microscopy discovered the considerably high motility of microglial processes [(Nimmerjahn et al., 2005); for detailed information on the historical context see (Tremblay et al., 2011)]. Meanwhile, high throughput gene expression analyses have started to decipher the origin and progeny of microglia (Prinz and Priller, 2014; Prinz et al., 2011) and their relevance in various physiological and pathological brain conditions (Butovsky and Weiner, 2018).

While the myriad roles of microglia in health and disease have been comprehensively reviewed by leading experts in the field [e. g., (Butovsky and Weiner, 2018; Kettenmann et al., 2013; Prinz and Priller, 2014)], this concise review article focuses on recent experimental evidence which suggests a fundamental role of microglia in modulating the ability of excitatory tripartite synapses to express plasticity (Figure 2). We will describe and discuss the emerging concept of the (quadpartite) neuroimmunological synapse and its implications in synaptic plasticity at the interface between health and disease.

Bidirectional interactions between microglia and neurons under physiological conditions

Based on structural and functional similarities between microglia and macrophages it was initially assumed that microglia exist in a resting state until pathological stimuli trigger their activation, e. g., proliferation, ameboid migration, phagocytosis and the release of inflammatory

cytokines. Meanwhile, a series of landmark studies has shown that microglia continuously survey the healthy CNS, with their processes getting close to pre- and post-synaptic compartments [Fig. 1*C*; (Nimmerjahn et al., 2005; Tremblay et al., 2011)] including axon initial segments (Baalman et al., 2015), i. e., all major structural and functional microdomains of neurons which generate, propagate or transmit signals.

These interactions are activity-dependent as a reduction in neural activity also reduces microglia dynamics (Li et al., 2012; Tremblay et al., 2010; Wake et al., 2009). Consistent with these observations, various neurotransmitter receptors, such as adrenergic, purinergic, glutamatergic and GABAergic receptors, are found on the surface of microglia which enables them to detect and respond to neurotransmitter release and changes in neural activity (Biber et al., 2007; Fontainhas et al., 2011; Pocock and Kettenmann, 2007).

In turn, microglia are known to mediate synapse formation and synaptic pruning during development (Paolicelli et al., 2011; Parkhurst et al., 2013; Wu et al., 2015), and they have been implicated in the modulation of excitatory and inhibitory synaptic transmission and plasticity [e. g., (Cantaut-Belarif et al., 2017; Pascual et al., 2012; Schafer et al., 2013)]. Interestingly, these physiological effects of microglia depend on signaling pathways traditionally studied in the context of neuroinflammation, e. g., complement and fractalkine systems (Bertollini et al., 2006), pro- and anti-inflammatory cytokines (Habbas et al., 2015), or partial phagocytosis (Weinhard et al., 2018). While the precise signals which recruit these neuroimmunological pathways under physiological conditions remain not well-understood, an indisputable activity-dependent interaction between microglia and neurons seems to exist, which is expected to play fundamental roles in complex brain function.

Tumor necrosis factor alpha (TNFα) mediates homeostatic synaptic plasticity and modulates the ability of neurons to express Hebbian plasticity

Among the best studied microglial factors that influence synaptic plasticity is the pro-inflammatory cytokine TNFα (Cahoy et al., 2008; Zhang et al., 2014). TNFα acts through two canonical receptors: TNF-receptor 1 (TNFR1)

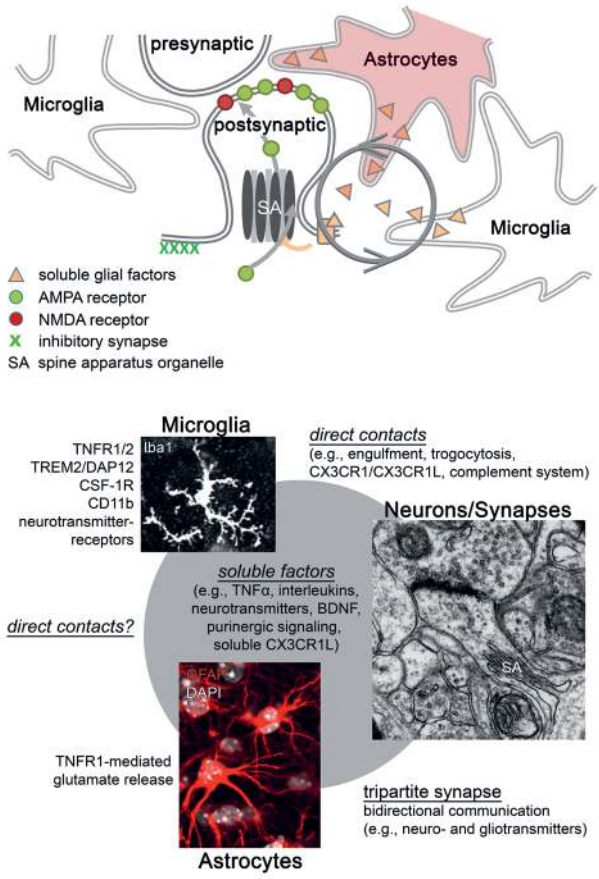

Figure 2: The (quadpartite) neuroimmunological synapse
Schematic illustration of structural and functional interactions between microglia, astrocytes and neuronal presynaptic and postsynaptic compartments (SA, spine apparatus organelle). Details provided in the text.

and TNF-receptor 2 (TNFR2). TNFR1 is activated by membrane-bound and soluble TNFα while TNFR2 predominantly binds to membrane-bound TNFα (Dopp et al., 1997; Probert, 2015). In the CNS both receptors are detected on neurons and glial cells. Hence, TNFα-signaling may account for both, microglia mediated secretion of TNFα (via TNFR1) and cell-cell interactions (via TNFR1/TNFR2) at synaptic sites (Figure 2).

Consistent with the observation that microglia assert physiological functions, TNFα has been linked to homeostatic synaptic plasticity (Stellwagen and Malenka, 2006), which is a form of plasticity that plays a fundamental role in maintaining physiological brain function. It was shown that a reduction of network activity – which reduces microglia motility (Wong et al., 2011) – leads to glial TNFα release (Barnes et al., 2017; Habbas et al., 2015; Stellwagen and Malenka, 2006). In turn, TNFα induces a compensatory increase in excitatory synaptic strength (Stellwagen and Malenka, 2006) which brings neurons back to their former activity state (Beattie et al., 2002; Stellwagen et al., 2005). While evidence exists that TNFα also downregulates inhibitory neurotransmission (Pribiag and Stellwagen, 2013), it may be important to note that in a recent study we were not able to detect homeostatic changes in inhibitory neurotransmission in a lesion model that is known to trigger glial activation and increased TNFα levels (Lenz et al., 2019). Thus, the precise role of microglia and TNFα in coordinating homeostatic plasticity of excitatory and inhibitory neurotransmission remains a matter of future investigations.

Meanwhile, it has been also suggested that TNFα may act as a permissive factor in the context of synaptic plasticity (Becker et al., 2013; Maggio and Vlachos, 2014; Steinmetz and Turrigiano, 2010). Hence, microglia may assert their effects on plasticity not by inducing changes in synaptic transmission and strength *per se*, but may rather act as neuromodulators: Through the release of TNFα microglia modulate the ability of neurons to express plasticity without necessarily affecting baseline synaptic transmission. Indeed, a recent study demonstrated that low concentrations of exogenously applied TNFα improve the ability of neurons to express excitatory synaptic plasticity, i. e., long-term potentiation (LTP) of Schaffer collateral-CA1 synapses, without affecting synaptic strength or previously established LTP in the same set of hippocampal slices (Maggio and Vlachos, 2018). Interestingly, high doses of TNFα had an opposite effect and impaired LTP – again not affecting baseline synaptic transmission and previously established LTP (Maggio and Vlachos, 2018). These results demonstrate that TNFα can act as a mediator of metaplasticity, i. e., it modulates the ability of neurons to express LTP in response to the exact same stimulus. Hence, it is conceivable that microglia surveille synaptic transmission and upon changes in neural activity (or yet unknown neuronal or astrocytic co-stimulatory factors) they can modulate the ability of synapses to express further plasticity depending on the concentrations of membrane-bound or locally secreted TNFα.

Microglia-mediated modulation of the tripartite synapse

What are the cellular and molecular targets through which microglial TNFα affects synaptic transmission and plasticity? A solid line of experimental evidence exists which suggests that TNFα can act on astrocytes, leading to an increase in glutamate-release by astrocytes (Habbas et al.,

2015; Santello et al., 2011). In turn, presynaptic NMDA-receptors will be activated which modulate presynaptic release properties. Indeed, evidence has been provided that astrocytic TNFR1 mediates this phenomenon, which could be relevant in various physiological and pathological conditions (Habbas et al., 2015).

With respect to postsynaptic mechanisms, our recent work identified the actin-binding molecule synaptopodin as a target of microglial TNFα (Maggio and Vlachos, 2018; Strehl et al., 2014). Synaptopodin is an actin-modulating protein enriched in a subset of dendritic spines [and in the axon initial segments; (Schluter et al., 2017)] of cortical principal neurons (Mundel et al., 1997; Deller et al., 2000). It is a marker and essential component of the spine apparatus organelle, an enigmatic cellular organelle composed of stacked smooth endoplasmic reticulum [(Deller et al., 2003); c.f., Figure 2], which regulates homeostatic plasticity and LTP via intracellular calcium stores [(Vlachos et al., 2013; Vlachos et al., 2009); for a recent review see Jedlicka and Deller, 2017]. Indeed, in absence of synaptopodin low concentrations of TNFα do not improve synaptic plasticity in our experimental setting (Maggio and Vlachos, 2018). Consistent with this observation, low concentrations of TNFα increase synaptopodin expression and the sizes of spine apparatus organelles [c.f., (Vlachos et al., 2013)], while high concentrations of TNFα are expected to reduce synaptopodin expression and impair hippocampal plasticity (Strehl et al., 2014). Although it remains to be shown whether these effects of TNFα are mediated by TNFRs on neurons (and not through an astrocytic mechanism), they support the notion that microglia affect plasticity by modulating structural and functional properties of tripartite excitatory synapses (Figure 2).

The term tripartite synapse refers to the functional interactions and structural proximity of neuronal (1) presynaptic, (2) postsynaptic membranes and (3) the surrounding astrocytic endfeet (Figure 2). Work from recent years has started addressing the functional significance of tripartite synapses in synaptic transmission/plasticity and complex behavior [e. g., (Chever et al., 2016; Dallerac and Rouach, 2016)]. Also considering the well-established role of inflammatory cytokines and other immune mediators in modulating synaptic plasticity, it has been proposed that microglial processes, which interact with tripartite synapses (Fig 1C), may constitute the forth compartment of a *quadpartite synapse* (Schafer et al., 2013). Because microglia assert their effects on synaptic plasticity via signaling pathways traditionally studied in the immune system the term (quadpartite) '*neuroimmunological synapse*' (c.f., Table 1) seems applicable in this context.

Relevance of microglia-mediated neuromodulation in the context of brain disease

Alterations in cognitive function and behavior are often observed in the context of neurological diseases associated with neuroinflammatory responses and/or infection of the central nervous system [e. g., (Heneka et al., 2018)]. As pointed out, immune mediators have been identified that affect synaptic plasticity (Werneburg et al., 2017). This is of considerable relevance in the context of neurological and psychiatric diseases associated with increased brain levels of pro-inflammatory cytokines (Heneka et al., 2018). Hence, microglia activation by endogenous or exogenous non-physiological stimuli are expected to disturb physiological interactions and homeostasis between microglia, astrocytes and neurons at *neuroimmunological synapses* eventually leading to alterations in synaptic plasticity.

The biological consequences of alterations in synaptic plasticity are not well-understood. Apparently, a microglia-mediated impairment of synaptic plasticity – as seen for example under conditions of high TNFα levels – cannot be simply interpreted as detrimental, since it is possible that a reduction in the ability of neurons to express synaptic plasticity protects neural networks from maladaptive changes. However, microglia-mediated alterations in synaptic plasticity may hamper functional recovery at a later stage of the disease. Considering the emerging concept of the *neuroimmunological synapse* and the well-established bidirectional interactions between neural activity and microglia function, a vicious cycle between pathological microglia activation and neural network alterations may arise, which could initiate, promote or sustain pathological brain states. It is tempting to speculate that exogenous (therapeutic) modulation of neural activity and plasticity could affect and potentially counteract the detrimental effects of neuroinflammation on *quadpartite* synapses, since microglia are known to respond to changes in neural activity.

In this context, repetitive transcranial magnetic stimulation (rTMS) may represent an interesting approach (Lefaucheur et al., 2014). Based on the physical principle of electromagnetic induction, TMS allows for the non-invasive stimulation of distinct cortical regions in awake and non-anesthetized human subjects and has been shown to modulate cortical excitability beyond stimulation [for review see (Lenz and Vlachos, 2016)]. Using an *in vitro* model of r(T)MS we recently demonstrated that repetitive magnetic stimulation induces plasticity of excitatory and inhibitory synapses (Lenz et al., 2015; Vlachos et al., 2012). The role of microglia in rTMS-induced plasticity has not been tested so far. Yet, it is conceivable that rTMS may provide an efficient approach to modulate structural and functional properties of *neuroimmunological synapses*, which may influence and even restore physiological microglia function under certain experimental conditions. It is tempting to speculate in this context that rTMS may also act on synaptopodin-associated calcium stores in dendritic spines and the axon initial segment. Regardless of these considerations, it is clear that a comprehensive understanding of the role of microglia in modulating synaptic plasticity will be important to identify new strategies for the treatment of brain diseases associated with microglia activation and neuroinflammatory responses.

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