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Snapshot of Microglial Physiological Functions

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Abstract

Microglia as a defensive arm of the nervous system emerged early in evolution. The surveilling microglia with motile and ramified processes are the main phenotype in the healthy CNS; the surveilling microglial patrol neuronal somata, dendrites, dendritic spines and axons. Increasing evidence suggests that microglia play fundamental roles in development, maturation and ageing of the brain, as well as contribute to a variety of physiological brain processes including sleep and circadian rhythm. Physiological state of microglia is tightly regulated by brain microenvironment and controlled by a sophisticated system of receptors and signalling cascades including ionotropic and metabotropic purinoceptors, pattern-recognition receptors, and receptors for chemokines and cytokines. Microglia also utilise ion channels and transporters in regulating ionic homeostasis and various aspects of microglial function. The major ion transporters expressed by microglia include Na^+/H^+ exchanger 1 and $\text{Na}^+/\text{Ca}^{2+}$ exchangers, which are involved in regulation of pH_i and Ca^{2+} homeostasis during microglial physiological responses. Microglial cells control development, maturation and plasticity of neuronal ensembles through controlled physiological phagocytosis of synapses or synaptic fragments - processes known as synaptic pruning and trogocytosis. This special issue on “Physiological roles of microglia” is an assembly of papers written by the leading experts in this research field. We start this special issue with this snapshot of microglial physiological functions as a prelude to the indepth discussion of microglia in physiological processes in the nervous system.

Key words: Microglia; Neuroprotection; Physiology; Synaptic pruning; Synaptic transmission; Receptors; Transporters

1. Microglia: neural cells of myeloid origin

Microglia as a defensive arm of the nervous system emerged very early in evolution; most notably main features of microglial cells from morphology to major gene clusters are conserved from medicinal leech to *Homo sapiens* (Geirsdottir et al., 2019). Microglia as “garbage collectors” of the central nervous system (CNS) have been discovered and characterised in great detail by Pio del Rio-Hortega, who documented a complex morphological transformations of these cells from ramified phenotype in the healthy brain to amoeboid phagocyte in pathology (Del Rio-Hortega, 1919, 1932; Sierra et al., 2016). In contrast to macroglia of ectodermal origin, microglial cells are alien to the brain: microglial origins are linked to foetal macrophages of the yolk sac that enters the neural tube at early developmental stages prior to the development of blood-brain barrier (Ginhoux et al., 2010; Stremmel et al., 2018; Swinnen et al., 2013). Immigration of macrophages into the neural tube follows a complex pattern, and the yolk sac may not be the only source for microglial precursors (Fehrenbach et al., 2018). Be this as it may, microglial precursors populate embryonic CNS and generate microglial cells dwelling in the adult brain throughout life; the self-renewal and microglial turnover seem to be self-regulated (i.e. not requiring exchange with circulating macrophages) in the healthy nervous system (Askew et al., 2017).

Microglial precursors disseminate throughout the CNS; their migration reaches the peak around birth with post-natal brains being engulfed with “fountains of microglia” (Kershman, 1939). These migrating precursors with amoeboid morphology populate all regions of the CNS and differentiate into surveilling microglia characteristic for the healthy nervous tissue (Kettenmann et al., 2011; Stratoulas et al., 2019; Tay et al., 2019; Wolf et al., 2017). The surveilling phenotype (also known as “resting” or “ramified” microglia” or “surveillant”) is highly idiosyncratic: the surveilling microglial cells have small cell body and several thin and long processes with terminal ramifications. These processes are in constant move (Nimmerjahn et al., 2005), scanning nervous tissue and establishing short-living contacts with various parts of neighbouring neurones, with their somata, axons and dendrites (Cserep et al., 2020a; Cserep et al., 2020b; Kettenmann et al., 2013; Tremblay et al., 2013). Microglial processes are endowed with an array of receptors represented by both immunocompetent receptors characteristic for macrophages and by receptors to neurotransmitters and neurohormones characteristic for neural cells (Doens and Fernandez, 2014; Kettenmann et al., 2011; Pocock and Kettenmann, 2007; Szepesi et al., 2018). Co-existence of two sets of receptors makes microglia, arguably, the most “receptive” cells in the CNS. The morphology and physiology of microglia differ fundamentally from peripheral macrophage, which represents a remarkable example of epigenetic metamorphosis of foetal macrophages that adapts them to a highly idiosyncratic environment of the nervous tissue (Garaschuk and Verkhratsky, 2019). Microglial receptors allow these cells to perceive neuronal activity and, at the same time, provide them with sensitivity to danger- and pathogen-associated molecular patterns (DAMPs and PAMPs) associated with pathology (Dasari et al., 2021). Adaptation of macrophages to CNS environment went further making microglial cells indispensable elements of neural networks. Microglial functions are not limited by defence and innate immunity; microglia are actively interacting with all cellular elements of the nervous

tissue and participate in numerous fundamental physiological functions of the CNS. In particular microglial cells contribute to homeostatic support of neurones, to shaping of neuronal ensembles through synaptic pruning and removal of apoptotic neurones during development, to synaptic transmission through release on various neuroactive molecules and to neuroprotection.

2. Microglial phenotypes

Microglial cells account ~5 - 6% of all cells in the CNS and they are distributed relatively homogeneously throughout brain regions (Dos Santos et al., 2020); although some variations have been also noted. For example, microglial densities are seemingly higher in the white matter (Mittelbronn et al., 2001; Savchenko et al., 2000). At the same time microglial population is heterogeneous with several distinct physiological phenotypes being described.

Amoeboid microglia of the developing brain are migrating microglial precursors which populate the brain immediately after birth. These cells have large somata with extremely small filopodia and they are capable of amoeboid movement (Haas et al., 1996; Tremblay et al., 2013). Amoeboid microglia settle down and acquire surveilling phenotype by postnatal day 10 – 15 in rodents.

The *surveilling microglia* is the main phenotype in the healthy CNS characterised by a small soma and motile processes. This active surveillance performed by moving processes is brain state-dependent: processes motility is reduced during wakefulness and increased during sleep (Liu et al., 2019). The mobility of microglial processes is a fundamental feature of these cells as it remains active even in conditions of metabolic stress (Szalay et al., 2016). Processes mobility under restricted metabolism can be sustained by utilizing glutamate as an energy substrate (Bernier et al., 2020). Microglial processes establish several distinct types of contacts with neural cells which reflect different patrolling modes.

Microglia-somatic patrolling represents a relatively long-lasting (up to 1 hours) contacts established between microglial process and neuronal soma. These microglia-somatic junctions have specific morphology; they are supported by purinergic signalling and P2Y₁₂ purinoceptors and are neuroprotective (Cserep et al., 2020b). Microglia-dendritic patrolling is represented by much shorter (~ 5 minutes) contacts between microglial processes and dendrites and especially dendritic spines. This type of patrolling is linked to synaptic pruning (Kettenmann et al., 2013; Sakai, 2020; Sierra et al., 2010). Microglia-axonal patrolling reflects intimate contacts established by microglial processes and axonal initial segments; these contact are reported to moderate axonal excitability thus preventing excitotoxicity (Baalman et al., 2015; Kato et al., 2016). Finally, microglial processes converging response represents the protective programme responding to minor lesions to the nervous tissue. Emergence of such lesions is associated with rapid increase in ATP deriving from damaged cells; ATP being an archetypal DAMP (Verkhatsky and Burnstock, 2014) activates microglial P2Y₁₂ receptors which instigate rapid converging

movement of microglial processes towards the site in lesion (Davalos et al., 2005; Haynes et al., 2006).

Satellite microglia discovered by Pio del Río-Hortega (Del Rio-Hortega, 1919) has cell bodies which directly contact apical and lateral regions of neuronal somata. In addition satellite extend a process along the axon-initial axonal (Wogram et al., 2016).

The **juxtavascular** or **perivascular** microglia (Gehrmann et al., 1995) extend processes toward blood vessels. These processes are terminated with the endfeet, which contribute to formation of *glia limitans vascularis* (Grossmann et al., 2002; Mondo et al., 2020).

3. Microglia physiology: receptors

The physiological state of microglia is tightly regulated by brain microenvironment; in particular a sophisticated system of receptors and signalling cascades controls microglial reactivity, which, if let loose, may cause substantial damage. Microglial state therefore is controlled by two distinct sets of signals - the "On" signals which trigger reactive microgliosis and the "Off" signals that are keeping microglial cells in their surveilling state (Biber et al., 2007; van Rossum and Hanisch, 2004). The On signals are represented by DAMPS and PAMPs, whereas "Off" signals are constitutively present in the healthy nervous tissue, being represented for example by neurotransmitters in physiological concentrations. Conceptually "Off" signals inhibit microglial reactivity; and their disappearance instigates microgliosis. All these signals are operating thorough multiple sets of microglial receptors.

Arguably the most prominent (and certainly the most studied) microglial receptors are represented by purinoceptors; which generally are widespread through all neuroglia (Timmerman et al., 2021; Verkhratsky et al., 2009; Verkhratsky et al., 2012). As alluded before, ATP, released from neurons and glia in physiological context and after damage (Abbracchio et al., 2009; Burnstock et al., 2011) acts as both classical neurotransmitter and archetypal, evolutionary conserved DAMP (Burnstock and Verkhratsky, 2009; Verkhratsky and Burnstock, 2014). As a consequence purinoceptors in pathology are acting as "On" receptors. Microglia express both ionotropic (mainly P2X₄ and P2X₇) and metabotropic (P2Y₂, P2Y₆, P2Y₁₂, and P2Y₁₃) purinoceptors. In pathological conditions, expression of microglial P2X₇ receptors is up-regulated and activation of P2X₇ receptors triggers microgliosis and secretion of pro-inflammatory factors (Kettenmann et al., 2011; Sanz et al., 2009). Furthermore, experimental over-expression of P2X₇ receptors in microglia instigates their reactive transformation even in the absence of any exogenous factors (Monif et al., 2009). The P2X₄ receptors are expressed in several subsets of microglia, in particularly in microglia of the spinal cord, where their pathological activation is linked to the neuropathic pain (Tsuda et al., 2003).

Microglial metabotropic purinoceptors are classical G-protein coupled receptors linked to phospholipase C and InsP₃ production which triggers Ca²⁺ release from the endoplasmic reticulum stores (Kettenmann et al., 2011). Activation of microglial metabotropic purinoceptors is often linked to the activation of secondary store-operated Ca²⁺ entry

(SOCE); excessive stimulation of these receptors results in long-lasting stimulation of SOCE, which may substantially modulate microglial reactivity (Toescu et al., 1998). Out of several types of P2Y receptors expressed by microglia, the P2Y₁₂ purinoceptors are of a specific importance. These receptors are expressed by surveilling microglia in the healthy nervous tissues (Haynes et al., 2006; Sasaki et al., 2003); they are concentrated in microglial processes and regulate various microglial patrolling modes (Cserep et al., 2020a; Lin et al., 2020)

Besides purinoceptors microglial cells express iono- and metabotropic receptors to glutamate (Kaindl et al., 2012; Noda et al., 2000; Yamada et al., 2006), GABA_B receptors (Kuhn et al., 2004), α_7 acetylcholine receptors, α_{1A} , α_{2A} , β_1 and β_2 adrenoceptors (Sugama and Kakinuma, 2020), D₁₋₄ dopamine receptors and 5-HT₂ serotonin receptors (Kettenmann et al., 2011). In addition, microglia express numerous receptors to neuromodulators and neurohormones including, for example, receptors to bradykinin, ET_B endothelin receptors, angiotensin receptors, somatostatin receptors, opioid receptors, and neurotrophin receptors to name but a few (Kettenmann et al., 2011).

From the immune heritage microglia retained expression of pattern-recognition receptors and receptors for chemokines and cytokines. Among pattern-recognition receptors, the Toll-like receptors (or TLRs) are ubiquitously expressed in microglial cells (Aravalli et al., 2007; Bsibsi et al., 2002; Ebert et al., 2007; Gurley et al., 2008; Timmerman et al., 2021). The TLR1/2, 6/2, 4 and 5 are mainly concentrated in the plasma membrane, whereas TLR3, 7, 8 and 9 are associated within endosomal compartments. The TLR1, TLR2 and TLR6, are activated by bacterial tri- and diacyl lipopeptides, lipoteichoic acid and peptidoglycan, the TLR3 is activated by virus-specific double stranded RNA, TLR4 is the receptor for bacterial cell wall component lipopolysaccharide (LPS), TLR5 is sensitive to bacterial flagellin, TLR7 and TLR8 are activated by viral RNA, whereas TLR 9 detects bacterial and viral unmethylated CpG DNA (Aravalli et al., 2007; Carpentier et al., 2008; Olson and Miller, 2004). As TLR4 expressed by microglia have been implicated in fear memory consolidation in posttraumatic stress disorder (Enomoto and Kato, 2021), TLRs may be involved in physiological process in the brain. Microglial cytokine receptors include TNF- α receptors, interleukin receptors and receptors to interferon β and γ . All these receptors are G-protein coupled and are linked to various signalling cascades such as mitogen-activated protein kinase (MAPK) or phosphatidylinositol-3 kinase (PI3-K) (Biber et al., 2008).

This remarkable set of receptors of various modalities makes microglia the perfect sentinels of brain environment; the patrolling microglia is capable of perceiving most physiological and pathological signals and mount an appropriate response.

4. Microglial physiology: solute carrier transporters

In addition to ionotropic neurotransmitter receptors, SOCE channels, microglia also utilise solute carrier (SLC) ion transporters in regulating ionic homeostasis and activation (Luo et al., 2021). The major ion transporters expressed by microglia include Na⁺/H⁺ exchanger 1 (NHE1), and Na⁺/Ca²⁺ exchangers (NCX). Operation of NHE1 maintains the

basal intracellular pH (pH_i) of microglia in resting conditions by extrusion of H^+ in exchange for Na^+ (Liu et al., 2010). Treatment with LPS or hypoxia stimulation triggers microglial respiratory burst and superoxide production by NADPH oxidase (NOX), which leads to intracellular H^+ accumulation and acidification (Liu et al., 2010). Activation of NHE1 in microglia is aimed at removing excess H^+ ions and maintain an optimal alkaline intracellular pH (pH_i) in supporting NOX activity (Lam et al., 2013; Liu et al., 2010; Shi et al., 2013a). The pH_i homeostasis is also important for microglial chemotaxis and migration (Shi et al., 2013b).

Microglia express all three isoforms of NCX, of which NCX1 is the most abundant (Newell et al., 2007; Parpura et al., 2016). The NCX1 plays a major role in regulation of Ca^{2+} in microglia, as silencing NCX1 with small interfering RNA (siRNA) completely abolishes Ca^{2+} signalling in cultured microglia after hypoxia (Boscia et al., 2013; Boscia et al., 2009). Reversal mode of NCX1 (NCX_{rev}) can be induced by inflammatory factors such as interferon (IFN)- γ in cultured microglia, which was mediated by upregulation of protein kinase C (PKC), tyrosine kinase, and MAPK/ERK (Nagano et al., 2004). The NCX1_{rev} -mediated Ca^{2+} influx is important for initiation of reactive microgliosis, migration, and phagocytosis after LPS stimulation in early postnatal mice (Sunkaria et al., 2016). However, the NCX1-dependent microglial migration is restricted to the bradykinin-induced chemotaxis, but not ATP-induced migration, as proven in the NCX1 knockout mouse model (Ifuku et al., 2007; Noda et al., 2013). In addition, NCX plays a critical role in microglial phagocytic activity through the Ca^{2+} -dependent activation of the purinergic receptors (P2Y_2 , P2Y_6 , and P2Y_{12}) during postnatal development (Sunkaria et al., 2016). Thus, NCX is important for mediating Ca^{2+} signalling in microglial activation. Microglial SLCs are mainly controlled by transmembrane gradient for Na^+ ions; operation of SLCs is accompanied with substantial Na^+ fluxes, and therefore, by analogy with astrocytes (Rose and Verkhratsky, 2016; Verkhratsky et al., 2020), microglial cytosolic Na^+ signalling can play important role in defining functional responses of these cells.

5. Microglial physiological functions

5.1. Synapse pruning in development

The surveilling microglia have been shown to engage in maturation of the neural circuits in the developing hippocampus (Paolicelli et al., 2011) or neural pathways in the visual systems (Schafer et al., 2012) by phagocytic elimination of synapses, known as synaptic pruning or synaptic stripping (Kettenmann et al., 2013; Tremblay et al., 2011). This contribution has been revealed by experiments using electron microscopy and/or two-photon *in vivo* imaging. The frequency of microglia-dendritic/synaptic contacts in ischemic murine brains depends on continuing activities of the degenerating synapses, favouring elimination of the pre-synapses (Wake et al., 2009). Microglia selectively engulf synapses in the lateral geniculate body in the thalamus that are derived from retinal ganglion cell when the activity of retinal ganglion cells was blocked with tetrodotoxin (Schafer et al., 2012). Microglial elimination of synapses is shown to be activity-dependent in the visual cortex of juvenile mice (Tremblay et al., 2010). Thus, the

synaptic elimination by microglia depends on the activity of neural circuit. Microglia express the specific marker CD11b, which is a part of complement C3 receptor. Microglia may recognise aberrant synapses that are tagged and opsonised with C3. The C3-tagged synapses are arguably glutamatergic because they express vesicular glutamate transporter 2 (Schafer et al., 2012). Neurone-microglia interactions through synapse pruning may be critical for the functional maturation of glutamatergic presynaptic functions (Basilico et al., 2019). Through elimination of the C3-tagged unnecessary synapses, microglia may play critical roles in the maturation of neural circuits in the developing brains (Konishi and Kiyama, 2020; Miyanishi et al., 2021). Mechanistically synaptic pruning relies of a specific type of *en passant* physiological (i.e. not associated with reactive microgliosis) phagocytosis which eliminates either whole synapses or their fragments; the latter process known as trogocytosis (Sakai, 2020; Sierra et al., 2010; Weinhard et al., 2018).

5.2. Synapse pruning in adult brain

Synaptic pruning by microglia may be at its maximum around at postnatal day 15 in the murine hippocampus (Weinhard et al., 2018). Nonetheless even in the adult healthy brain, microglia engulf and eliminate weak or redundand synapses that are tagged with "eat-me-signal" complement proteins and/or milk fat globule-EGF factor 8 protein (MFG-E8) (Brown and Neher, 2014). Sensory deprivation by removing whiskers causes apoptotic changes in synapses in the primary sensory cortex; these synapses are tagged with complement C1q that signals to microglial process and instigates synapse elimination (Brown and Neher, 2014). As described above, microglial processes display higher motility during the sleep compare to the awake state (Liu et al., 2019). In accordance with this observation, microglia phagocytose synapses tagged with complement or MFG-E8 during sleep (see Figure 1 and (Choudhury et al., 2020). Microglia eliminate not only weak but also hyperactive glutamatergic synapses that are present in the Parkinson's disease (PD) model brain (Aono et al., 2017). In the PD brain, loss of dopaminergic neurons leads to hyperactivity of glutamatergic neurons in the subthalamic nuclei; as a result, "excitatory transmission" becomes dominant. However, microglia eliminate hyperactive excitatory glutamatergic synapses from the subthalamic nuclei and the excitatory/inhibitory imbalance is somewhat rectified. Thus, microglia may contribute to amelioration of the motor symptoms of PD. This is one example for the role of microglia in the homeostasis in the neurotransmission.

Microglia seem to phagocytose only presynaptic elements (Weinhard et al., 2018). Furthermore, microglia may preferentially eliminate glutamatergic synapses bein less prone to phagocyte GABArgic synapses (Miyanishi et al., 2021). Therefore, impairment of microglial synaptic elimination may cause the imbalance between excitatory and inhibitory synaptic transmission, resulting in CNS pathology. Impaired synaptic elimination by microglia in the mature brain have been implicated in behavioural, psychiatric, and neurologic disorders as well as in disturbances of memory and sleep (Deurveilher et al., 2020; Miyanishi et al., 2021; Nakanishi et al., 2021). For example, autism spectrum disorder may be correlated with reduced synaptic pruning by microglia. Increased synaptic pruning is suspected as one of the pathophysiological causes for

schizophrenia. Microglial elimination of synapses in the adult hippocampus lead to degradation of memory engrams and enhancement of forgetting of previously learned contextual memory (Wang et al., 2020).

5.3. Modulation of synaptic activity

Microglia are also known to interact with ongoing synaptic transmission through secreting several chemical factors (Kettenmann et al., 2011). Ablation of microglia cause functional changes at both pre- and postsynaptic levels; these changes included decrease in expression of vesicular glutamate transporters with subsequent decrease in glutamate release as well as impaired expression of post-synaptic receptors. All these changes were attributed to the decrease in microglial BDNF secretion (Parkhurst et al., 2013). Similarly, microglial secretion of nerve growth factor (NGF) sustains glutamatergic transmission through activation of presynaptic TrkA receptors (Rizzi et al., 2018). Microglia can regulate synaptic transmission through release of exosomes, which contain anandamide; the latter was reported to reduce GABAergic transmission through activation of presynaptic CB-1 receptors (Gabrielli et al., 2015). Genetic elimination of microglial fractalkine CX3CR1 receptor results in aberrant development of glutamatergic transmission (Basilico et al., 2019; Zhan et al., 2014). Stimulation of CX3CR1 receptors with CX3CL1 induces adenosine-dependent signalling that impacts on glutamatergic transmission (Lauro et al., 2008; Ragozzino et al., 2006) acting through presynaptic adenosine receptors (Di Angelantonio et al., 2015; Piccinin et al., 2010). Microglia may also increase presynaptic glutamate release through secretion of IL-1 β that acts through postsynaptic NMDA receptors and retrograde messenger signalling (Clark et al., 2015). Microglial secretion of BDNF regulates inhibitory transmission through activation of neuronal TrkB receptors with subsequent down-regulation of Cl⁻ transporter KCC2 and aberrant Cl⁻ homeostasis (Coull et al., 2005). Resulting changes in Cl⁻ transmembrane gradient increases excitability of nociceptive neurones thus contributing to neuropathic pain (Mapplebeck et al., 2016).

5.3. Neuroprotection

In addition to protecting appropriate brain functions by synaptic pruning or scavenging of cellular debris, microglia also secrete trophic factors such as cytokine IL-1 β , IGF1, BDNF, etcetera (Tay et al., 2017) to promote normal brain functions. For example, deletion of the unique subpopulation of microglia in the subventricular zone decreased the survival and migration of newly generated neuroblasts to the olfactory bulb (Ribeiro Xavier et al., 2015). Preventing microglial release of BDNF decreased the formation of cortical dendritic spines during motor learning (Parkhurst et al., 2013). Knockout of microglial CX3CR1 or microglial depletion in mice resulted in cognitive impairment (Parkhurst et al., 2013; Rogers et al., 2011).

6. Conclusions

Taken together, microglia are multitasking (migration, phagocytosis of debris, pruning synapses, secretion of cytokines and trophic factors) to maintaining normal brain

homeostasis and functions. Imbalanced functions or dysregulation of microglial functions contribute to brain pathology development or hindered brain tissue repair in brain diseases. This Special Issue contains reviews of frontier microglial research in the broad spectrum including microglia in physiological brain functions such as sleep (Deurveilher et al., 2020), circadian rhythm regulation (Nakanishi et al., 2021), brain development and brain ageing (Konishi and Kiyama, 2020), as well as in brain pathologies such as neurodevelopmental and psychiatric disorders (Kakinuma, 2020; Miyanishi et al., 2021), stress-induced depressive disorders (Enomoto and Kato, 2021; Kikutani et al., 2020; Sugama and Kakinuma, 2020), acute brain injury (hemorrhagic and ischemic stroke (Dasari et al., 2021; Luo et al., 2021)) and brain tumours (Timmerman et al., 2021). These reviews illustrate the significance of understanding microglial functions and identifying modulators as therapeutic targets for treating brain disorders.

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Figure legends

Figure 1. Phagocytic elimination of synapses by microglia.

Microglia in the frontal cortex (layer II) of normal 8-week-old Wistar rats actively phagocytose synaptophysin-positive synapses around at zeitgeber time 0 when sleeping period for rats starts. (a) Microglia appear to eliminate only synapses that are tagged with "eat-me-signals" such as a complement C3. (b) The phagocytosed synapses are present in the CD68⁺ phagosomes in the Iba1⁺ somata of microglia.

Figure 1