

## Review

# Shaping Neuronal Fate: Functional Heterogeneity of Direct Microglia-Neuron Interactions

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## SUMMARY

The functional contribution of microglia to normal brain development, healthy brain function, and neurological disorders is increasingly recognized. However, until recently, the nature of intercellular interactions mediating these effects remained largely unclear. Recent findings show microglia establishing direct contact with different compartments of neurons. Although communication between microglia and neurons involves intermediate cells and soluble factors, direct membrane contacts enable a more precisely regulated, dynamic, and highly effective form of interaction for fine-tuning neuronal responses and fate. Here, we summarize the known ultrastructural, molecular, and functional features of direct microglia-neuron interactions and their roles in brain disease.

## INTRODUCTION

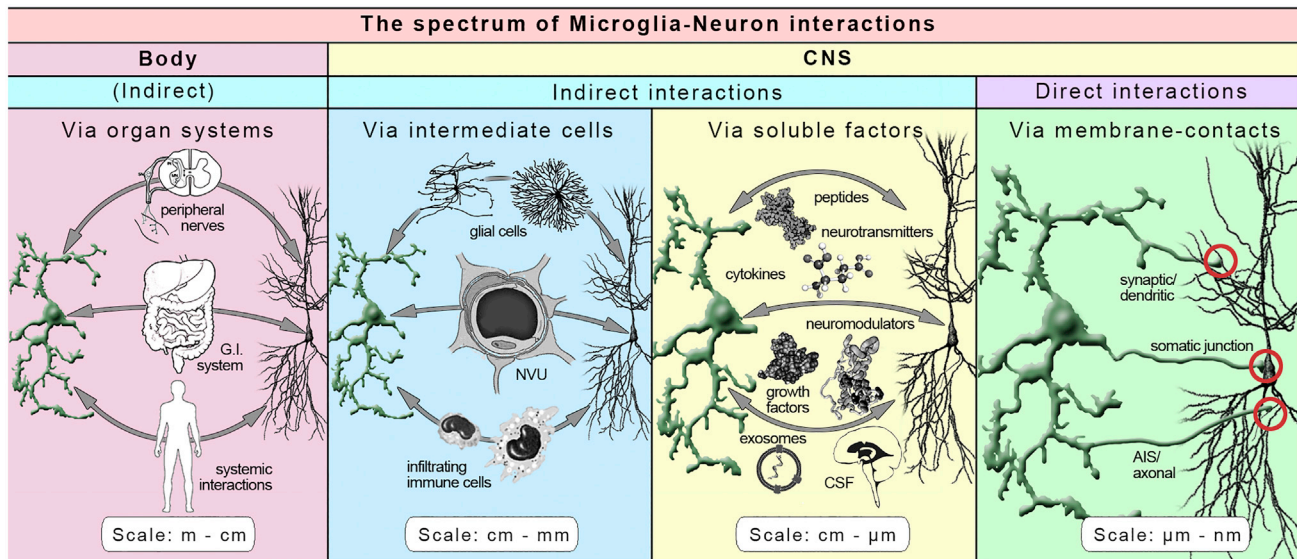
Our lack of understanding about how the normal brain functions and why its precise machinery becomes dysfunctional in brain diseases has substantially increased interest in glial biology in recent years. In line with this, marked improvement in the identification and targeting of glial populations fueled by the development of new molecular biological approaches has paralleled the emergence of high-resolution imaging tools and more advanced experimental models of neurological disorders. As a result, microglia, once considered another macrophage that becomes an amoeboid phagocyte upon injury, has emerged as a multifunctional housekeeping cell type with important roles in normal brain development, healthy brain function, and brain disorders.

Several fundamental questions in neuroscience remained unresolved based on merely considering interactions between neurons. These include synapse formation, synaptic pruning (elimination of excess synapses during development or in adulthood), spatial organization and integration of neuronal signals, brain metabolism, brain aging, and importantly, brain disorders (Südhof, 2017). Emerging data indicate that microglia play a previously unrecognized role in many of these processes from early embryonic development to advanced age. For example, microglia contribute to embryonic neurogenesis (Cunningham et al., 2013), neuronal differentiation (Aarum et al., 2003), developmental and activity-dependent synaptic pruning (Paolicelli et al., 2011; Schafer et al., 2012), synapse elimination upon sensory lesioning (Gunner et al., 2019), formation of cortical layers (Ueno et al., 2013), adult neurogenesis (Diaz-Aparicio et al., 2020; De Lucia et al., 2016; Sellner et al., 2016), removal of apoptotic neurons (Sierra et al., 2010), learning-dependent synapse formation (Parkhurst et al., 2013), synapse and spine remodeling (Weinhard et al., 2018), and axonal guidance (Pont-Lezica et al., 2014; Squarzone et al., 2014). In addition, microglia not only undergo

marked changes during aging (Grabert et al., 2016) but also emerge as important contributors to several age-related conditions, including common neurological disorders (Angelova and Brown, 2019; Marschallinger et al., 2020; Spittau, 2017).

To understand how microglia work, the great diversity of microglial phenotypes (changes during development and aging, region-dependent heterogeneities in the brain, etc.) (Masuda et al., 2020a), their functional responses, and the broad range of interactions between microglia and other cells is important to study, but integration of knowledge emerging from these studies is also instrumental. Many resources elaborate on microglial function and interactions with neurons (Izquierdo et al., 2019; Kierdorf and Prinz, 2017; Szepesi et al., 2018; Wilton et al., 2019). However, only recent advances in modern imaging tools, genetic models, and photochemical approaches have enabled more precise visualization of such interactions *in vivo* (Akiyoshi et al., 2018; Damisah et al., 2020; Masuda et al., 2020b). In line with this, developments in molecular anatomy have revealed previously unknown complexity of membrane-membrane interactions that emerge as key determinants in how microglia recognize injured neurons, shape neuronal activity, or determine the fate of salvageable and terminally injured cells (Akiyoshi et al., 2018; Cserép et al., 2020; Diaz-Aparicio et al., 2020; VanRyzin et al., 2019; Weinhard et al., 2018). These interactions manifest at different temporal and spatial scales; therefore, unless they are studied extensively, it remains difficult to understand their impact on brain health and disease. For example, considering that individual microglia may live for years (Réu et al., 2017), a single microglial process that touches a dendritic spine or the soma of a neuron 2–6 times in 1 h will establish around half a million contacts within a decade. The frequency of such interactions provides innumerable occasions not only to monitor and foster neuronal functions but also to cause harm if the delicately regulated balance of these interactions is disturbed.





**Figure 1. The Spectrum of Microglia-Neuron Interactions**

Indirect, long-distance interactions between microglia and neurons establish complex regulatory loops that include remote actions of microglial processes on neurons in peripheral tissues. In turn, circulating mediators or peripheral nerves shape microglial function. Such interactions occur during diverse forms of neuroimmune communication in health and disease. Within the CNS, indirect microglial interactions can involve communication via soluble factors and intermediate cells. These interactions may employ signaling via other glial cells (oligodendrocytes and astrocytes), cells of the neurovascular unit, or infiltrated immune cells. Indirect interactions via soluble factors (cytokines, growth factors, neuromodulators, etc.) also provide means of intercellular communication with long-range effects via the cerebrospinal fluid (CSF) or with shorter-range effects in the parenchyma limited by the diffusion and clearance of the active mediators in the extracellular space. Direct microglia-neuron interactions via tight membrane-membrane contacts permit the most effective, rapid, and dynamic communication between the two cell types. The scales are only referring to an approximate range of effect. (Drawing of the neuron is courtesy of Attila Gulyas, with permission; Megias et al., 2001.)

In this review article, we focus on the fine-tuned intercellular communication between microglia and neurons that requires great proximity of the two cellular membranes, called direct interactions throughout this paper. We summarize the known ultrastructural, molecular, and functional properties of different microglia-neuron interactions and then discuss the functional role of microglial actions in different physiological and pathological processes throughout life.

### The Spectrum of Microglia-Neuron Interactions

Communication between microglia and neurons takes place on multiple levels, ranging from indirect interactions through soluble messengers to direct membrane-to-membrane contacts (Figure 1), with more sophisticated regulation of both microglial and neuronal functions toward the reduction of distance between the two cell membranes. In this section, we follow a top-down approach to give a general overview of the spectrum of microglia-neuron interactions.

#### Indirect Interactions via Organ Systems

Indirect, bidirectional microglia-neuron interactions may be distant and can span the body, involving several transmitting stations (Figure 1). As such, microglia may shape the activity of neurons in the central nervous system (CNS) or peripheral nerves via neuronal or humoral routes, whereas microglial actions may be influenced by neurons in the CNS or in the peripheral nervous system (Benakis et al., 2020; Sharon et al., 2016; Thion et al., 2018). For example, microglia-neuron interactions influence peripheral immune processes via autonomic nerves or soluble fac-

tors (Andersson and Tracey, 2012; Chu et al., 2020; Norris and Kipnis, 2019). Altered cerebrovascular function or microglial activity leads to perturbations of the microbiota, which in turn exerts its effect on CNS neurons (Ma et al., 2019; Sharma et al., 2019; Singh et al., 2016). In parallel with this, microorganisms of the gastrointestinal tract affect systemic inflammatory processes (Fung et al., 2017) that shape neuronal activity and neuroinflammation (Dantzer et al., 2008; Gyoneva et al., 2014), including their effects on microglial activity (Streit et al., 2004).

#### Indirect Interactions via Intermediate Cells

Within the CNS, information can be indirectly passed from neurons to microglia, and vice versa, through various intermediate cells. For example, astrocytes simultaneously receive and provide information that shapes the responses of both neurons and microglia (Allen and Eroglu, 2017; Matejuk and Ransohoff, 2020). Neurons continuously exchange metabolites with astroglia (Roman et al., 2020; Roosterman and Cottrell, 2020; Zulfiqar et al., 2019), whereas the tight collaboration among microglia, astrocytes, and neurons has been revealed in the quad-partite synapse (Schafer et al., 2012) and during development (Vainchtein and Molofsky, 2020), among others. In the neurovascular unit, endothelial cells, pericytes, or smooth muscle cells are also involved in the complex and unexplored signaling between microglia and neurons, whereas bidirectional interactions between microglial cells and brain vasculature are essential during both development and adulthood (Haruwaka et al., 2019; Zhao et al., 2018). In response to brain injury, microglia influence the recruitment of peripheral immune cells into the brain (Fekete

et al., 2018; Otxoa-de-Amezaga et al., 2019; Unger et al., 2018), whereas effects of immune-cell-derived mediators (e.g., proteases and cytokines) on neuronal activity and injury are well documented (Filiario et al., 2017; Gadani et al., 2015; Ortega et al., 2020).

#### **Indirect Interactions via Soluble Factors**

Intercellular communication via soluble factors, which often exert their effects distant from their source, enables modulation of larger populations of cells, but in a less target-specific manner, compared with direct cell-cell interactions. Microglia-derived mediators, such as brain-derived neurotrophic factor (BDNF) (Coull et al., 2005; Parkhurst et al., 2013), interleukin (IL)-10 (Lim et al., 2013; Pereira et al., 2015), IL-1 $\beta$  (Hewett et al., 2012; Huang et al., 2011), or tumor necrosis factor alpha (TNF- $\alpha$ ) (Beattie et al., 2002; Olmos and Lladó, 2014), alter neuronal activity. Recently, it has also been shown that neuronal ATP triggers microglial adenosine production to regulate neuronal responses (Badimon et al., 2020). Neurons also signal toward microglia using soluble mediators like purinergic metabolites (Davalos et al., 2005) or glutamate (Dissing-Olesen et al., 2014; Eyo et al., 2014; Fontainhas et al., 2011). The differences between the long-range and the short-range effects of most soluble mediators in the context of microglia-neuron interactions are unknown. Therefore, different forms of direct interactions between microglia and neurons, as discussed later, are likely to include known effects mediated by soluble factors, which may also be released from opposing cell membranes or vesicles at direct microglia-neuron contact sites.

#### **Direct Interactions**

Throughout the body, direct membrane-membrane interactions among cells enable sophisticated and well-controlled forms of communication. Good examples are the dynamic cell-cell contacts that govern the activation and effector function of peripheral immune cells, such as those formed between T cells and antigen-presenting cells (Freiberg et al., 2002; Garcia et al., 1996; Torralba et al., 2019). Communication that occurs through membrane protein interactions on opposing surfaces triggers intracellular signaling cascades that lead to the formation of an immunological synapse, which comprises a spatiotemporally regulated supramolecular cluster of proteins at the interface between the cells. These interactions are precisely controlled: key aspects of immune cell surfaces, including reorganization of signaling proteins at the contacts, can be even modeled using synthetic membranes or vesicles (Biggs et al., 2011; Jenkins et al., 2018). In the CNS, well-known forms of membrane-membrane communication include interactions between neurons via synaptic membranes or gap junctions, dynamic intercellular crosstalk within the syncytial network of astrocytes, interactions between astrocyte processes/endfeet and synapses or endothelial cells, and metabolite exchange between the myelin sheet and the axon. These interactions establish localized and functionally distinct signaling to control different biological processes (Droz, 1979; Giaume et al., 2010; Gray, 1959; Hamada and Kole, 2015; Kuffler et al., 1966; Stassart et al., 2018; Ventura and Harris, 1999).

Microglia are constantly monitoring the brain parenchyma with their motile processes under physiological conditions (Nimmerjahn, 2005), and neuronal activity is confirmed to be a driver of

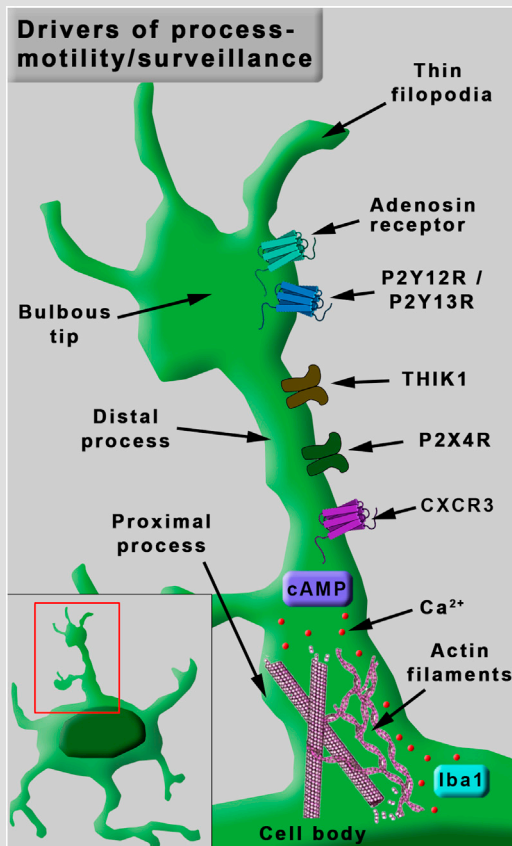
microglial process motility (Akiyoshi et al., 2018; Umpierre et al., 2020). The exceptionally high energy consumption of the brain makes it instrumental to function as efficiently and economically as possible. Thus, the maintenance of constant movement of microglial processes must have a substantial motive. One logical reason can be the apparent need to establish direct membrane-membrane contacts with other brain cells (see Box 1). Because the translocation of microglial cell bodies in the healthy brain is several orders of magnitude slower (Eyo et al., 2018) than the movement of microglial processes (Sipe et al., 2016), dynamic membrane-membrane contacts are preferentially established by the latter ones. Although microglial cell bodies are also capable of making direct contact with neurons, called satellite microglia, this type of interaction could be a less dynamic and more ancient form of intercellular communication (Baalman et al., 2015; Wogram et al., 2016) that resembles the more primitive satellite glial cells in the peripheral nervous system, whose direct contact with neurons has been implicated in delayed and noisy information transfer between these cells (Rozanski et al., 2013). Therefore, here we focus on the direct interactions between microglial processes and different neuronal compartments based on the available data. We first cover the idea of neuronal compartmental heterogeneity and then move on to the anatomical, molecular, and functional assessment of direct microglial interactions with different neuronal compartments. Finally, we provide an outlook on the presumed uncharted areas and future directions of this expanding research field.

#### **The Emerging Concept of Compartment-Dependent Functional Segregation of Microglia-Neuron Interactions**

Among all cell types, neurons possess probably the most complex and most extended three-dimensional (3D) morphology, with an extensive number of highly branched processes (Misgeld and Schwarz, 2017). This diverse and elaborate architecture may provide bases for the connectivity patterns and determines the functional properties of neurons, both of which are needed for proper divergence, convergence, transmittance, processing, and storage of information within neuronal networks. Neuronal geometry underlies universal network features in cortical microcircuits (Gal et al., 2020), neuronal architecture determines emergent cortical activity (Nolte et al., 2020), permits and restricts the connectivity and plasticity in neuronal networks (Chklovskii, 2004; Reimann et al., 2017). Furthermore, synaptic and dendritic morphologies per se determine neuronal information processing (Harnett et al., 2012; Holderith et al., 2012), and the complex morphology of neuronal cells is a strong predictor of their electrophysiological properties (Michiels, 2020).

Although neurons are frequently considered quasi-discrete and unitary computational units, compartment-dependent segregation of certain neuronal functions exists within their elaborate structures (Donato et al., 2019). The formation of new and the elimination of unwanted synapses, synaptic scaling through different plasticity mechanisms, branch formation, noise filtering, and signal integration are all mainly associated with dendritic arborization of neurons (Jan and Jan, 2010; Makino and Malinow, 2011; Südhof, 2018; Südhof and Malenka, 2008;

**Box 1. Microglial Surveillance and Process Heterogeneity as Drivers of Membrane-Membrane Interactions**



Pioneering *in vivo* two-photon imaging studies revealed that microglia constantly survey the brain microenvironment with their motile processes (Davalos et al., 2005; Nimmerjahn, 2005). Although the precise energy demand of this activity is unclear, there is a likely evolutionary advantage in keeping microglia working in the brain. In fact, surveillance activity is maintained even in the injured brain, such as after cerebral ischemia or brain trauma (Ohsawa and Kohsaka, 2011; Szalay et al., 2016), enabling microglia to respond to diverse pathophysiological stimuli even if the brain's energetic homeostasis is compromised. This results from their remarkable metabolic flexibility, for example, the ability to consume glutamine as an alternative metabolic fuel in the absence of glucose (Bernier et al., 2020).

Motility is achieved via organization of the actin cytoskeleton that is coupled with a sensory system of membrane receptors, allowing microglia to perceive changes in their microenvironment and modulate their responses (Franco-Bocanegra et al., 2019). This complex workout requires proteins involved in actin bundling and membrane ruffling, such as ionized calcium binding adaptor molecule 1 (Iba1); receptors that sense chemotactic signals, such as CX3CR1, or purinergic receptors, including P2Y12R, P2X4R, or P2Y13R; ion channels, such as TH1K-1, that regulate microglial membrane potential and receptor signaling; mechanisms that allow ion exchange between the cell and the extracellular matrix (e.g., via P2Y2R); and anchoring structures such as  $\beta$ 1-integrin (Haynes et al., 2006; Hristovska and Pascual, 2016; Madry et al., 2018a).

Microglial process motility and surveillance are precisely controlled, although the underlying mechanisms remain to be established. Large-scale regulatory mechanisms, such as noradrenergic control of baseline microglial surveillance activity (Liu et al., 2019; Stowell et al., 2019) or neuronal activity (Nebeling et al., 2019; Umpierre et al., 2020), exert constant modulatory effects on process motility by interfering with cell-intrinsic mechanisms (e.g., microglial membrane potential). Although

ATP release appears to be essential for injury-induced microglial process recruitment via P2Y12R, constant surveillance of the brain by microglia was found to be unaffected in the absence of P2Y12R signaling (Haynes et al., 2006; Sipe et al., 2016). In line with this, a two-pore domain  $K^+$  channel, TH1K-1, which is tonically active in the absence of P2Y12R activation, was shown to maintain microglial resting potential, ramified morphology, and normal surveillance activity (Madry et al., 2018a). The findings that no ambient purinergic signaling is required *in vitro* to maintain microglial ramification and surveillance were strengthened by a lack of detectable ambient ATP/ADP in brain slices and by blockade of ecto-ATPase activity not influencing these responses (Madry et al., 2018b). These original observations become more difficult to interpret once heterogeneity of microglial processes (either anatomically or functionally) and effects of the microenvironment on the movement of individual microglial processes are considered. It is likely that individual microglial processes respond to local cues that may overwrite cell-intrinsic mechanisms that control process extension and membrane ruffling.

Such functional segregation may be provided by the heterogeneity of microglial processes, which is supported by the observations that calcium dynamics of microglial processes and endfeet appears to be largely autonomous from the cell body (Umpierre et al., 2020). At the end of highly motile, large processes, microglia form actin-dependent filopodia that allow fast, nanoscale sensing of their environment (Bernier et al., 2019). These structures work in opposition to large processes: formation of filopodia is induced by increasing intracellular cyclic AMP (cAMP), whereas activation of  $G_i$ -coupled P2Y12Rs collapses filopodia, triggering extension of large processes with bulbous tips (Bernier et al., 2019). In addition, individual processes are likely to respond to different cues depending on their exact location. For example, activity-dependent ATP release from the neuronal somata regulates microglial process recruitment and coverage of the cell via P2Y12R that has been demonstrated after blockade of P2Y12R by PSB0739 injected into the cisterna magna (Cserép et al., 2020). Thus, compartment-dependent effects of P2Y12R on microglial process responses are likely to work in parallel with the molecular pathways regulating microglial morphology, process motility, and surveillance. These will collectively regulate the formation of membrane-membrane interactions between microglia and neurons.

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**Box 1. Continued**

The functional autonomy and heterogeneity of microglial processes could also explain how processes of the same microglial cells could monitor and communicate with multiple compartments of several different neurons or even different cell types. Membrane-to-membrane signaling at direct microglia-neuron contact sites and the surrounding chemical milieu are likely to simultaneously influence the nature of these interactions (e.g., responses to phagocytic or pro-apoptotic signals, excitotoxicity, etc.). This way, under healthy conditions, communication and regulatory processes could take place in a confined space, like process-specific calcium dynamics (Umpierre et al., 2020), and upon noxious stimuli or in case of injury, when a certain threshold is exceeded, the activity could be extended to the whole microglial cell. For example, whereas process-specific calcium signaling in microglia could mediate functionally heterogeneous responses during interactions with different cell types or cellular compartments under physiological conditions, depolarization-induced decrease in microglial surveillance (Madry et al., 2018b) could occur in diverse pathological states and is likely to shape responses of microglia to subsequent stimuli. Still, to date, we have limited information about microglial integration of the various signals they receive. Whether microglial signal integration, similar to that of neurons, involves the temporal information of afferent stimuli and the surrounding extracellular milieu is yet to be investigated.

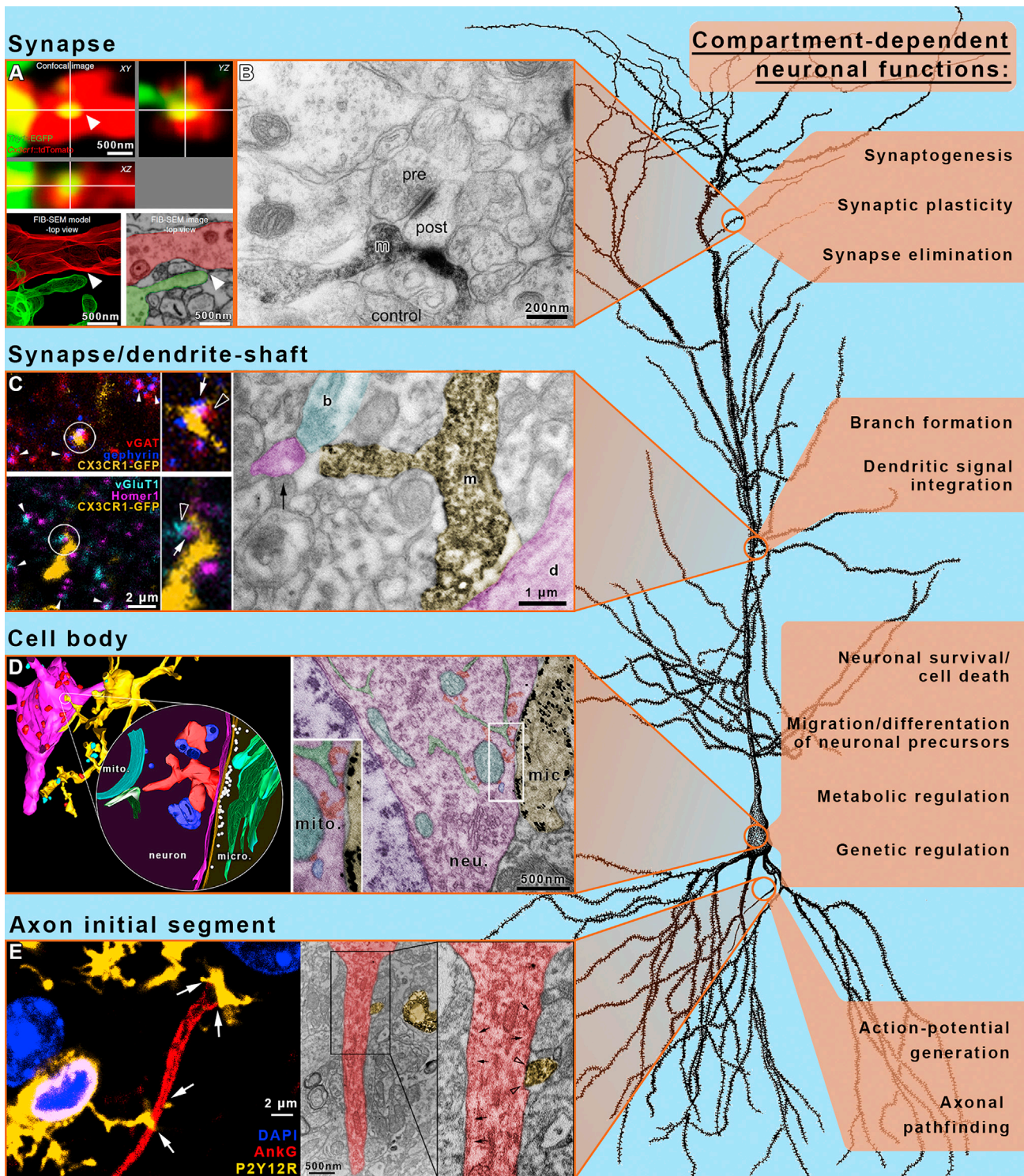
Terenzio et al., 2017). The storage of genetic material, genetic regulation, transcription, regulation of cellular metabolism, cell fate decisions involving differentiation of neuronal precursors, cell survival and death, and regulation of cell migration are fundamentally linked to the cell body (somatic compartment) of neurons (Aarum et al., 2003; Hobert et al., 2010; Marín-Teva et al., 2011; Sierra et al., 2014; Ueno et al., 2013). Generation of action potentials, gating of output signals and conducting of action potentials are executed by the axon initial segment (AIS) and axons (Bender and Trussell, 2009; Clark et al., 2009; Debanne et al., 2011; Huang and Rasband, 2018; Leterrier, 2016, 2018; Salzer, 2003; Suminaite et al., 2019).

In agreement with the previously mentioned division of labor, subneuronal compartments possess a high degree of functional independence concerning metabolism, protein synthesis, and signal integration. Metabolic autonomy of different subcompartments depends on mitochondrial dynamics, mainly mitochondrial trafficking and structural plasticity. Recent studies suggest that the contribution of mitochondrial motility is significantly smaller than previously thought (Lewis et al., 2016; Smit-Rigter et al., 2016), whereas locally regulated plasticity of mitochondria is more prominent, because an overwhelming body of evidence confirms that dynamic ultrastructural remodeling of mitochondria takes place as a response to altered energetic demand (Cogliati et al., 2016; Cserép et al., 2018). The importance of these processes, collectively called mitostasis, is highlighted by an elegant review (Misgeld and Schwarz, 2017). Local protein translation in dendrites, spines, and even axons is also fundamental for proper neuronal function (Biever et al., 2019; Cioni et al., 2018), adding a further level to the subcompartments' functional autonomy. Neuronal signaling is also highly compartmentalized, requiring sophisticated mechanisms to convey and integrate information within and between subneuronal compartments (Terenzio et al., 2017). We can also assume the existence of a hierarchy concerning the stability of different neuronal compartments. The cell body of neurons is relatively stable under most conditions in the adult brain, whereas axonal/dendritic branches can undergo activity- and environment-dependent changes, with synapses—the most dynamic structures—subject to continuous changes through different plasticity mechanisms (Bosch and Hayashi, 2012; Citri and Malenka, 2008; Williams

et al., 2007). Altogether, compartment-specific segregation of neuronal functions provides tempting opportunities for motile microglial processes to shape neuronal actions in a compartment-dependent manner. In line with this, metabolic, signaling, and regulatory independence of these compartments and their distinct stability are likely to require microglia to interact with subneuronal compartments differently. As discussed later, emerging evidence supports the concept of compartment-dependent functional segregation of microglia-neuron interactions, taking an important step away from the overly generalized view of intercellular communication between these cells. Although many underlying molecular pathways are unexplored, we suggest that direct microglial contact with different neuronal compartments serves different roles, depending on the compartment-specific neuronal functions and the compartment-specific signaling pathways (subcellular/molecular composition) involved (Figure 2).

### Anatomical, Ultrastructural, and Molecular Properties of Direct Microglia-Neuron Interactions Synaptic/Dendritic Interactions

The most studied interaction site between microglial processes and neurons is the microglia-synapse interface. Direct membrane-membrane interaction between microglial processes and synaptic boutons and spines has been confirmed using electron microscopy (Tremblay et al., 2010; Wake et al., 2009). Microglial processes contacting dendritic shafts of neurons have also been observed using *in vivo* 2-photon imaging (Miyamoto et al., 2016), confocal laser scanning microscopy (CLSM), and electron microscopy (Cserép et al., 2020). An elegant study by Weinhard et al. (2018), using correlated CLSM and scanning electron microscopy, demonstrated that microglia preferentially contact presynaptic elements, rather than dendritic spines; however, not all appositions (observed with CLSM) represent direct membrane-membrane contacts verified by electron microscopy (Figure 2). Using specific pre- and postsynaptic markers, 9% of glutamatergic and 11% of  $\gamma$ -aminobutyric acid (GABA)-ergic synapses were found to be apposed by microglial processes (Cserép et al., 2020) in perfusion-fixed brain tissues. However, relatively little is known about the possible involvement of subcellular organelles



**Figure 2. Types of Direct Microglia-Neuron Contacts Based on Neuronal Compartments**

(A) Confocal laser scanning microscopy (CLSM) of Thy1:EGFP/CX3CR1:tdTomato mouse shows the close apposition of a neuronal dendritic spine (green) and a microglial process (red). The two profiles apparently make contact, but the high-resolution 3D reconstruction from focused ion-beam scanning electron microscopy confirms the lack of direct contact, showing that electron microscopy is indispensable for the verification of direct cell-cell interactions. (With permission from Weinhard et al., 2018.)

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(mitochondria, synaptic or dense core vesicles, multivesicular bodies, etc.) in intercellular communication at these sites. Although several molecular mechanisms have been identified in functional studies to be involved in microglia-neuron communication at synapses—the complement system; the fractalkine-fractalkine receptor pathway; CD200-CD200R, DAP12-TREM, CD47-CD172a, and C3-CD11b interactions; etc. (Hong et al., 2016; Pósfai et al., 2019; Szepesi et al., 2018; Wilton et al., 2019; Zhang et al., 2018)—only a limited number of markers have been localized at these connections using specific immunolabeling and high-resolution microscopy. The complement component 1q (C1q), a key protein for microglia-dependent synapse elimination, was shown to be localized to developing synapses using CLSM and immunofluorescent labeling, together with synaptic markers PSD95 and SV2 (Stevens et al., 2007). Complement component 3 (C3) was shown to occasionally colocalize with the synaptic marker Homer1 (Shi et al., 2015b). CD47, a transmembrane protein that protects synapses from excess microglia-mediated pruning, has also been shown to localize to presynaptic boutons using CLSM and immunofluorescent labeling of the presynaptic vGluT2 and postsynaptic Homer1 markers (Lehrman et al., 2018). Major histocompatibility complex class I (MHC class I) molecules have also been shown to colocalize with PSD95 and be involved in the regulation of synaptic plasticity (Shatz, 2009).

#### **Perisomatic Microglia-Neuron Interactions and Somatic Purinergic Junctions**

Microglial processes have previously been implicated in the stripping of cell bodies from perisomatic axon terminals (Kettenmann et al., 2013; Trapp et al., 2007; Tremblay et al., 2010). Microglial process recruitment near highly active neuronal cell bodies has also been observed in the developing zebrafish optic tectum, which is densely packed with neuronal cell bodies (Li et al., 2012), and in the mouse cerebral cortex (Szalay et al., 2016). However, these observations did not report the prevalence, ultrastructure, molecular identity, and function of direct interactions between the two cells. We recently demonstrated the formation of somatic purinergic junctions between microglial processes and specialized membrane domains of neuronal cell bodies, which were extensively characterized and included a detailed ultrastructural-molecular analysis (Cserép et al., 2020). Microglial

processes have been found to contact the cell bodies of most neurons independent of the brain area and neurochemical phenotype in both mice and humans. These morphofunctional units possess a specialized ultrastructural and molecular composition optimized for dynamic intercellular communication (Figure 3). Neuronal mitochondria, mitochondrion-associated membranes (MAM), endoplasmic reticulum-plasma membrane contacts, vesicle-like structures, vesicular nucleotide transporter (vNUT) and lysosomal-associated membrane protein 1 (LAMP1) are all enriched and localized to these junctions, whereas exocytosis-promoting Kv2.1 and Kv2.2 proteins (binding SNARE elements) are clustered in the neuronal membrane within the contact sites. Microglial P2Y12 receptors (P2Y12Rs) were found to specifically accumulate in contacting microglial membranes within the junctions, unlike at perisomatic boutons, indicating the involvement of purinergic mechanisms in somatic junction formation. Transmission electron tomography confirmed the presence of discrete intercellular tethers between microglial processes and neuronal somatic membranes within these junctions.

#### **AIS/Axonal Direct Contact**

Microglial processes are also recruited to AISs, as shown by using immunolabeling for the specific marker Ankyrin-G (Baalman et al., 2015). The direct membrane-membrane contact was described only after traumatic brain injury (Benusa and Lafrenaye, 2020), whereas our unpublished data confirm the presence of direct contact between microglial processes and intact AIS of hippocampal neurons in the healthy brain (Figure 2). From a molecular point of view, fractalkine-fractalkine receptor interactions, complement-mediated processes, and DAP12-TREM signaling have all been connected functionally to microglia-axon interactions, but specific localization of the key molecular elements at these sites remains to be established (Figure 3).

#### **Overview of the Compartment-Dependent Functional Properties of Direct Microglia-Neuron Interactions and Their Implications in Health and Disease Synaptic/Dendritic Interactions**

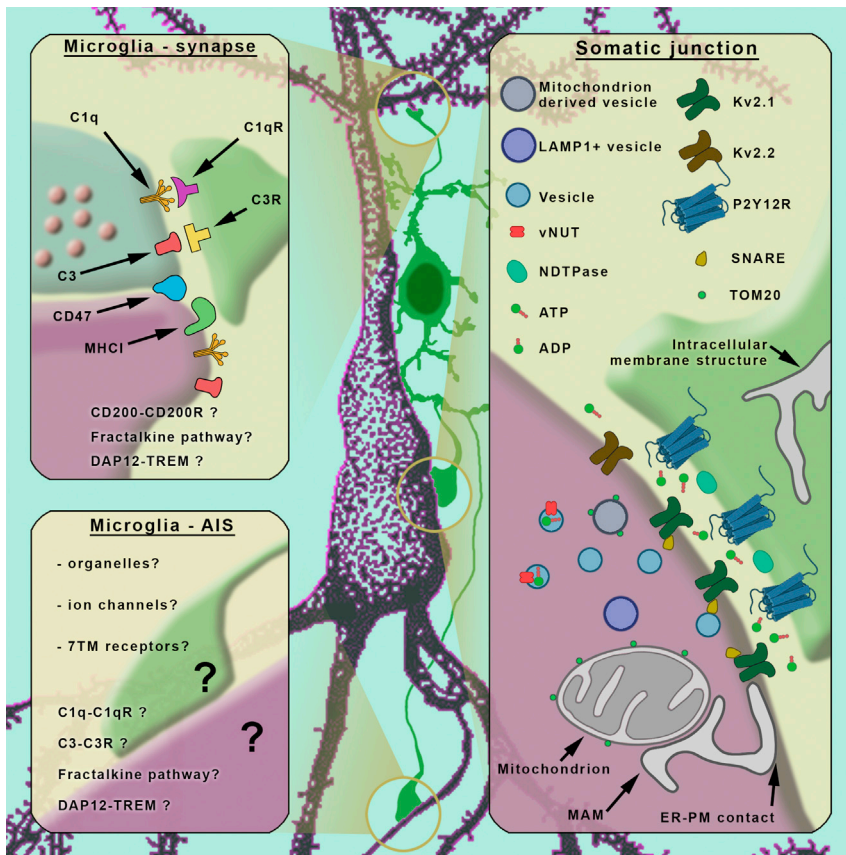
Connections between motile microglial processes and synapses are unequivocally the most studied interactions between microglia and neurons. Formation of these contacts has been shown

(B) Transmission electron micrograph shows direct contact of an ionized calcium binding adaptor molecule 1 (Iba1)-diaminobenzidine (DAB)-labeled (dark precipitate) microglial profile with both the pre- and the postsynaptic sides of a glutamatergic synapse in the mouse cerebral cortex. (With permission from Wake et al., 2009. Copyright 2009 Society for Neuroscience.)

(C) CLSM images show CX3CR1-GFP-positive microglial processes (yellow) to come into close apposition with both GABAergic (labeled for vesicular GABA transporter and gephyrin, red and blue) and glutamatergic synapses (labeled for vesicular glutamate transporter 1 and Homer1, cyan and magenta) in the mouse cerebral cortex. Transmission electron micrograph shows direct contact between an Iba1-DAB-labeled (dark precipitate, m) microglial process and a presynaptic bouton (cyan pseudocolor, b) of a glutamatergic synapse (arrow points to dendritic spine, magenta pseudocolor) and a dendrite shaft (magenta pseudocolor, d). (With permission from Cserép et al., 2020.)

(D) Left: 3D reconstruction of a confocal volume scan showing a microglial cell (yellow) establishing direct contact with the cell body of a cortical pyramidal cell (magenta), as well as with GABAergic (red) and glutamatergic (cyan) synapses. The insert shows the electron tomographic reconstruction of a somatic microglial junction. Note the specific ultrastructure on the neuronal side (membrane, magenta) with a nearby mitochondrion (turquoise), mitochondrion-associated membrane (pale green), and reticular (red) and vesicular (blue) organelles. The microglial membrane (yellow) is in direct contact with the neuronal membrane, and microglial P2Y12Rs (white) are concentrated around the core of the somatic junction. Right: electron microscopic image shows a microglial process (mic, yellow) establishing direct membrane-to-membrane contact with the somata of a neuron (neu, magenta). A mitochondrion (mito, turquoise) and anchored reticular and vesicular organelles are visible. Black grains mark anti-P2Y12R immunogold particles. (With permission from Cserép et al., 2020.)

(E) CLSM image shows microglial processes (P2Y12R, yellow) wrapping around an AIS (Ankyrin-G, red) in the mouse cerebral cortex. Arrows point to putative contact sites. A transmission electron micrograph shows direct contact between an Iba1-DAB-labeled (dark precipitate, yellow pseudocolor) microglial process and an AIS (red pseudocolor). Empty arrowheads mark the edges of the contact site; black arrows point to the dark undercoating, a prominent ultrastructural feature of AIS. (Unpublished material, DenesLab; the drawing of the neuron is courtesy of Attila Gulyas, with permission; Megias et al., 2001.)



**Figure 3. Compartment-Dependent Structural and Molecular Heterogeneity of Microglia-Neuron Contacts**

Microglia-synapse contacts have been shown to express elements of the complement system, namely, complement component 3 (C3) and complement component 1q (C1q). Markers of glutamatergic synapses also colocalize with CD47 and MHC class I molecules. Although the CD200-CD200R pathway, the fractalkine pathway, and DAP12-TREM signaling have also been strongly implicated as players in microglia-synapse interactions, their localization is not yet confirmed at these sites. Somatic junctions possess a specialized structure: the neuronal cytoplasm is enriched in anchored mitochondria, mitochondrion-associated membranes (MAMs), endoplasmic reticulum-plasma membrane (ER-PM) contacts, and purinergic and mitochondrion-derived vesicles (MDVs). Vesicular nucleotide transporter (vNUT) and the lysosomal marker LAMP1 are also enriched at the contact sites, whereas exocytosis-promoting Kv2.1 and Kv2.2 proteins (binding SNARE proteins) are clustered in the neuronal membrane within the contact sites. Accumulation of microglial P2Y12Rs is seen in contacting microglial membranes, together with NDTase molecules that may allow ATP hydrolysis to be focally controlled by microglial processes. The expression of CD47 could also be observed at these contacts. Microglia-AIS contacts represent the lesser known area of direct microglia-neuron interactions, because no subcellular organelle or molecular element has been shown to be specifically localized at these contacts. (Drawing of the neuron is courtesy of Attila Gulyas, with permission; Megias et al., 2001.)

to be activity dependent (Akiyoshi et al., 2018; Wake et al., 2009) and essential for the existence and maturation of synapses. Microglia-synapse and microglia-dendrite contacts usually persist for a few minutes *in vivo* (Cserép et al., 2020; Wake et al., 2009). During development, microglia contribute to the formation of neuronal circuits by regulating synaptic pruning (Paolicelli et al., 2011; Schafer et al., 2012; Weinhard et al., 2018) and synaptic development via the fractalkine receptor (Hoshiko et al., 2012; Paolicelli et al., 2011; Zhan et al., 2014) and complement signaling (Schafer et al., 2012). The precise mechanisms for how this occurs and the nature of membrane-membrane contacts required for these interactions remain to be established. Nevertheless, annexin V and local phosphatidylserine externalization have been shown to be necessary for developmental synaptic pruning (Scott-Hewitt et al., 2020). Microglial contact is also known to induce calcium-dependent spine formation of layer 2/3 pyramidal neurons in the developing somatosensory cortex (Miyamoto et al., 2016), indicating that contacting microglial processes are likely to establish rapid and localized signaling at defined areas of active dendrites. During adulthood, microglial processes participate in activity-dependent spine remodeling (Parkhurst et al., 2013) and synapse elimination (Trapp et al., 2007; Tremblay et al., 2010; Yamada et al., 2008) and regulate synaptic scaling, plasticity, postsynaptic currents, and local network synchrony by direct contact (Akiyoshi et al., 2018; Pfeiffer et al., 2016; Rogers et al., 2011; Wang et al., 2020). Purinergic metabolites like ATP, together with microglia-derived me-

diators such as TNF- $\alpha$ , IL-33, or IL-1 $\beta$  have been shown specifically to influence synaptic scaling, postsynaptic current frequency, or synaptic plasticity (Clark et al., 2015; Nguyen et al., 2020; Pascual et al., 2012; Stellwagen and Malenka, 2006).

Microglia play an important and highly complex role in regulating synaptic plasticity and network synchronization. Microglial colony-stimulating factor 1 (CSF-1) and BDNF have been shown to be essential for long-term plasticity in the spinal cord (Zhou et al., 2019); BDNF also modulates the expression of specific glutamatergic receptor subunits (Parkhurst et al., 2013). TNF- $\alpha$  regulates AMPA receptor-mediated currents and receptor trafficking (Lewitus et al., 2014; Stellwagen et al., 2005). *In vitro* recordings suggested a role for the fractalkine pathway in depressing excitatory currents (Ragozzino et al., 2006) and evoking long-term potentiation (Rogers et al., 2011). Noradrenergic signaling through microglial  $\beta_2$  receptors inhibits physiological plasticity processes (Stowell et al., 2019). Modification of dendritic spines may also take place through microglial reorganization of extracellular matrix (Tremblay and Majewska, 2011) or direct contact with spines that results in elevated neuronal network synchrony (Akiyoshi et al., 2018). This latter function is lost upon microglial activation by lipopolysaccharide (LPS) (Akiyoshi et al., 2018), which under hypoxic conditions also leads to hippocampal long-term depression through microglial CR3 signaling (Zhang et al., 2014).

Phagocytic clearance of excess or apoptotic synapses by microglia is an important homeostatic feature of the brain. Multiple



pathways have been implicated in this process: apoptotic synapses may express phosphatidylserine to induce microglial clearance through MFG-E8/integrin  $\alpha_v\beta_3$  or TAM receptors (Nonaka and Nakanishi, 2019), and synapses to be erased can be tagged with C1q, which initiates microglial eradication of labeled synapses in a CR3-dependent manner (Schafer et al., 2012; Stevens et al., 2007). Meanwhile, microglial trophocytosis of presynaptic boutons does not require CR3 (Weinhard et al., 2018). In parallel, MHC class I molecules expressed in or near synapses also regulate synapse elimination and plasticity (Elmer et al., 2013; Goddard et al., 2007; Lee et al., 2014) that could involve neuroimmunological actions. Phosphatidylserine externalization was found mostly presynaptically (Scott-Hewitt et al., 2020), which could explain the microglial preference to phagocytose the boutons instead of the spines (Weinhard et al., 2018).

The perturbation of this sensitive, strictly regulated interplay between microglia and synapses has been implicated in aging, different forms of tauopathies, and neurodegenerative conditions, including Alzheimer's disease (AD). The accumulation of C1q in the aging or diseased brain or synaptic damage mediated by Tau accumulation leads to pathological synaptic pruning by microglia that can be prevented by inhibiting the complement cascade (Hong et al., 2016; Rajendran and Paolicelli, 2018; Stephan et al., 2013; Vogels et al., 2019). Microglia-associated genetic risk factors (e.g., TREM2 and CD33) of AD and observation of an increased amount of phagocytosed synaptic material in post-mortem human AD samples point to the essential role of microglia in pathology (Malik et al., 2015; Tzioras et al., 2019). Interactions between microglial processes and synapses are also decisive for synapse elimination following cerebral ischemia (Wake et al., 2009), whereas disturbance of synaptic pruning may lead to various neuropsychiatric disorders, altered neuronal functional connectivity, and social abnormalities (Jung and Chung, 2018; Wang et al., 2018; Zhan et al., 2014). The importance of microglia-synapse interactions is also highlighted by a finding showing that Zika virus infection causes synapse loss and memory deficits via complement-dependent synapse elimination by microglia (Figueiredo et al., 2019). However, formation and regulation of the molecular assemblies that govern the preceding interactions at the microglia-synapse interface, and their alterations in different forms of neuropathologies remain to be defined.

### **Perisomatic Microglia-Neuron Interactions and Somatic Purinergic Junctions**

The interactions between microglia and neuronal synapses or dendrites do not explain how microglia are capable of monitoring and influencing the activity of neurons over months or even years or how their interactions may determine the outcome of complex physiological and pathological processes from development to aging. For example, it is likely that migration, proliferation, differentiation, and survival of neural precursors, as well as neurogenesis during development and in adulthood, require specific interactions between microglia and neuronal somata (Aarum et al., 2003; Bilimoria and Stevens, 2015; Erblich et al., 2011; Ueno et al., 2013). Microglia reportedly contribute to survival of cortical neurons during postnatal development (Ueno et al., 2013); integration of newly born neurons into the existing circuits; elimination of supranumerous, apoptotic neurons (Kettenmann

et al., 2013; Sierra et al., 2010; VanRyzin et al., 2019); and regulation of adult neurogenesis via CX3CR1 (Sellner et al., 2016).

Microglia-neuron communication with perisomatic neuronal compartments has been observed in studies investigating microglial regulation of neuronal activity or experimental models of brain diseases, although it remained unclear until recently whether the mechanisms identified are functionally linked with interactions between microglia and neuronal somata or between microglia and perisomatic synaptic terminals, or simply represent bystander changes with microglia-synapse interactions. Previous studies showed that in zebrafish and in mouse cortical brain slices, microglia-neuron crosstalk was associated with reduction of neuronal hyperactivity or neurotoxicity (Eyo et al., 2014; Li et al., 2012). Activity-dependent microglial process outgrowth and interactions with neurons were found to require NMDA receptor signaling (Dissing-Olesen et al., 2014; Eyo et al., 2014). P2Y12Rs, which are specific for microglia in the brain and regulate chemotaxis of microglial processes to ATP/ADP, have been implicated in different neurophysiological processes. Selective elimination of microglia or pharmacological blockade of microglial P2Y12Rs was found to reduce neuronal calcium overload after experimental stroke (Cserép et al., 2020; Szalay et al., 2016); P2Y12R blockade also altered seizure-induced neurogenesis (Mo et al., 2019), the innate fear response (Peng et al., 2019), or neuronal activity in chronic neuropathic pain (Gu et al., 2016). It is unclear how and through which neuronal compartments microglia mediate these effects, but data have shown that microglia-neuron interactions at somatic purinergic junctions are functionally linked with changes in neuronal activity (Cserép et al., 2020); therefore, the role of somatic microglia-neuron crosstalk in controlling neuronal excitability- and hyperexcitability-associated injury is likely.

Based on the long lifetime (~25 min on average), the molecular architecture, and the functional properties of somatic microglial junctions, these interactions may allow constant monitoring of neuronal well-being by microglia, which may include the detection of early events of cellular injury, regulation of cell survival and programmed cell death, and phagocytosis of damaged neuronal cell bodies. In fact, neuronal activity, mitochondrial function, and exocytosis appear to be functionally linked with the formation of somatic microglial junctions (Cserép et al., 2020). Clustering of exocytosis-promoting neuronal Kv2.1 was found to be important for microglial contact formation *in vitro*, whereas activity-dependent somatic ATP release from neurons depended on vNUT (enriched in neurons at somatic junctions) and L-type  $Ca^{2+}$  channels (involved in somatic vesicle release). Proximity of mitochondria and changes in mitochondrial NADH in neurons were linked to microglial contact formation and intact P2Y12R function. Chemogenetic neuronal activation or neuronal injury promoted increases in microglial process coverage of neuronal somata at the junctions in a P2Y12R-dependent manner. In line with this, neuronal injury was associated with breakdown of Kv2.1 clusters and mitochondrial fragmentation, whereas increased excitotoxicity and brain damage were found in response to experimental brain injury when microglia-neuron communication was blocked by acute P2Y12R inhibition (Cserép et al., 2020). Similarly, an absence of microglia was associated with increased  $Ca^{2+}$  load and triggered slow neuronal

**Box 2. Communication between Neuronal Mitochondria and Microglia: Legacy of the Mutual Past**

Mitochondria are intracellular biological engines responsible for a plethora of critically important cellular functions. These functions extend beyond the canonical duty of energy production and include a range of signaling and housekeeping tasks, such as regulation of calcium fluxes, cell proliferation, migration, cell morphology, and viability (Chandel, 2014; Whelan and Zuckerbraun, 2013). Researchers generally agree that mitochondria were acquired by endosymbiosis more than 1.45 billion years ago (Gray et al., 2001; Martin and Mentel, 2010; Roger et al., 2017), although there are data pointing to an even earlier origin (Martijn et al., 2018). Because of their bacterial ancestry and subsequent similarities with these prokaryotic pathogens (Boguszewska et al., 2020), the innate immune system had to develop tolerance toward mitochondria (Rongvaux, 2018). Despite the development of this tolerance, the immune system keeps a close eye on these versatile organelles, with multiple ancient communication channels remaining alive (Breda et al., 2019; Jin et al., 2017; Soltys and Gupta, 2000). As such, mitochondria serve as immunometabolic hubs (Bantug et al., 2018; Weinberg et al., 2015) that are also enriched at immunological synapses and involved in antigen presentation (Maccari et al., 2016; Matheoud et al., 2016).

Microglia, the resident immune cells of the CNS, are responsible for a vast amount of homeostatic and regulatory functions during development and normal brain physiology (Kierdorf and Prinz, 2017; Nimmerjahn, 2005), extending beyond the stereotypical immune-cell-related roles (Salter and Stevens, 2017). The origin of microglia can be traced back at least to the emergence of leeches (Hartenstein and Giangrande, 2018; Pósfai et al., 2019; Verkhratsky et al., 2019). Because neuronal stress is sensed by microglia via many possible routes, including ATP release, proapoptotic signals, and ROS, that have strong association with mitochondrial function in neurons and other cells, microglia are likely to monitor diverse mitochondrial clues that remain to be identified.

Mitochondria are not only master regulators of danger signaling (Galluzzi et al., 2012) but also can provide perfect readout of cellular functions (Gao and Zhang, 2018; Sugiura et al., 2014), making them ideal candidates to be the status reporters of their parent cells. Microglial process recruitment can be driven by multiple signals, such as purinergic metabolites, extracellular cytochrome c, ROS, glutamate, DAMPs, PAMPs, mtDNA, and apoptotic signals (Bajwa et al., 2019; Davalos et al., 2005; Eyo et al., 2014; Galluzzi et al., 2012; Gouveia et al., 2017; Gülke et al., 2018), many of which are mitochondrial related. The idea of mitochondrion-microglia communication is even more prominent in the light of the newly described somatic microglia-neuron junctions (Cserép et al., 2020), in which neuronal mitochondria are enriched and anchored to the neuronal plasma membrane. This compact intracellular assembly provides an ideal site for release of mitochondria-associated messenger molecules to be constantly sensed by microglia (see the main text). Because the activity of mitochondria at somatic junctions also depends on microglial contact formation (Cserép et al., 2020), these mitochondria can readily communicate neuronal status toward microglia and may be influenced by microglial interactions. Similar mitochondria-driven interactions may also occur between microglia and other cell types.

oscillations in the ischemic penumbra, leading to increased neuronal death (Szalay et al., 2016).

We propose that healthy neurons may constitutively release ATP (Cserép et al., 2020; Zhang et al., 2007) and other signaling molecules at somatic microglial junctions, reflecting their state toward microglia, whereas disintegration of these specialized morphofunctional hubs caused by excitotoxicity, energy depletion, or other noxious stimuli may trigger rapid microglial responses, leading to the restoration of neuronal function or elimination of terminally injured cells. Microglia may continuously monitor neuronal status through multiple, mitochondria-related signaling pathways (e.g., via release of ATP, mitochondria-derived vesicles, proapoptotic signals, and markers of oxidative stress). Although somatic mitochondria are key determinants of the energetic/metabolic balance and the immunological status of neurons and other cells (Gao and Zhang, 2018; Weinberg et al., 2015), mitochondria-MAM interfaces—frequently associated with somatic junctions—are complex controlling stations of neuronal functions in health and disease (Csordás et al., 2018). Besides providing a reasonable readout of cellular state, somatic mitochondria represent an effective controlling interface of neuronal functions, including metabolism, firing threshold, calcium homeostasis, proliferation, and cell-fate decisions (Chandel, 2014; Hall et al., 2012; Kasahara and Scorrano, 2014; Rugari and Langer, 2012; Styr et al., 2019; Tepikin, 2018), which microglial actions may interfere with. Interestingly, mitochondria-mi-

croglia communication observed at these junctions might have a coevolutionary origin (see Box 2). Disturbed mitochondrial function in neurons is seen alongside altered microglial activity in the early stages of common neurological disorders (Arun et al., 2016; Lezi and Swerdlow, 2012; Politis et al., 2012; Shen et al., 2018). As such, altered mitochondrial function, known to be present in Parkinson's disease (PD) or AD (Joshi and Mochly-Rosen, 2018), may trigger alterations at somatic microglial junctions, which will need to be investigated in the future.

Phagocytosis of dead cells occurs during developmental programmed cell death or during the elimination of apoptotic or necrotic cells because of acute brain injury, neurodegeneration, or infection (Fekete et al., 2018; Janda et al., 2018; Sierra et al., 2010). Similar to the effective control of synapse dynamics by microglia (Hong et al., 2016), discrimination of salvageable neurons from irreversibly injured cells is vital, because prolonged exposure to dead cells could trigger excessive inflammation. Considering that aging, neuroinflammation, neurodegeneration, and systemic inflammatory changes simultaneously affect neuronal activity, injury, and microglial phenotypes (Chen et al., 2016; Norden and Godbout, 2013; Sankowski et al., 2015), the accuracy of microglial target recognition needs to rely on precisely controlled membrane-membrane interactions. Soon after signals from dying or dead cells are detected, microglial processes are rapidly recruited, followed by dislocation of the cell body, eventually leading to ensheathment and phagocytosis of neuronal cell

bodies. It has recently been shown that microglia and astrocytes eliminate different parts of apoptotic neurons, which takes place via precisely regulated cell-cell interactions. Dendrites, cell bodies, and nuclei of apoptotic neurons are phagocytosed primarily by microglia, whereas astrocytes engulf diffuse apoptotic bodies derived from the extensive dendritic arbors without dislocation of their cell body (Damisah et al., 2020). Among the large number of damage-associated molecules, including HMGB1, DNA, and histones (Roh and Sohn, 2018), ATP is a well-known chemotactic molecule for microglial processes that is released from injured cells (Cook and McCleskey, 2002; Davalos et al., 2005; Haynes et al., 2006). The contribution of P2Y12R to the recognition and phagocytosis of apoptotic or virus-infected neurons by microglia has also been demonstrated (Diaz-Aparicio et al., 2020; Fekete et al., 2018). Somatic microglial junctions therefore appear to be ideal sites for microglia to rapidly identify dying cells (e.g., based on the release of mitochondria-derived signals or altered ATP release, followed by activation of membrane receptors upon contact formation) and initiate phagocytosis. Once membrane-to-membrane contacts occur between microglia and neurons, removal of dead cells requires the presence of membrane receptors, such as those from the TAM family, including the receptor tyrosine kinase Merck that senses translocated phosphatidylserine on the outer membrane of dying cells (Damisah et al., 2020; Diaz-Aparicio et al., 2020).

#### **AIS/Axonal Direct Contact**

During brain development, neuronal axons grow and find their targets through a complex and precisely regulated process to establish the desired connectivity patterns. Although gradients of soluble factors contribute fundamentally to this process, direct interactions between microglia and axons or axonal growth cones are more than likely to be involved in its regulation. Microglia are essential for the proper formation of the corpus callosum (Pont-Lezica et al., 2014), whereas microglial cells accumulate at decision points along specific axonal tracts, acting as definite guidepost cells (Squarzone et al., 2014). During postnatal development, microglia also contact axons of projection neurons and contribute to their survival (Fujita et al., 2020). However, direct microglial contact with growth cones was also shown to inhibit axonal outgrowth (Kitayama et al., 2011) and to be instrumental for developmental fiber reorganization (Dalmau et al., 1998). In the adult brain, microglial processes migrate toward the axons of hyperactive neurons and repolarize their membrane (Kato et al., 2016). Microglial clearance of degenerating axons is also contributing to regeneration (Bechmann et al., 2001; Neumann et al., 2009), whereas pro-regenerative microglia is also important for remyelination (Lloyd et al., 2019). Microglial interactions with the AIS appear early in development, persist throughout the lifespan, and are conserved across species (Baalman et al., 2015). Microglia also contact diffusely injured axons after traumatic brain injury (Lafrenaye et al., 2015), and microglial contact is implicated in disturbed AIS integrity in experimental autoimmune encephalomyelitis (Clark et al., 2016). The AIS receives an important GABAergic synaptic input from the chandelier cells (Szentágothai and Arbib, 1974), and the possible three-way interactions among microglial processes, the AIS, and axo-axonal boutons can add another level of complexity to this intercellular communication.

#### **Outlook/Uncharted Areas/Future Directions**

Several open questions need to be addressed to understand the key mechanisms of compartment-specific microglia-neuron communication and its role in different diseases of the brain. This will require the integration of functional data with molecular anatomy of the microglia-neuron interactome at different neuronal compartments. Considering the highly controversial roles for microglia in experimental models of different neurological diseases (Chen et al., 2018; Gomes-Leal, 2012; Qin et al., 2019), compartment-specific actions by microglia could partly explain why bulk interventions may have different effects in given pathophysiologies. For example, whereas genetic deletion of the fractalkine receptor (CX3CR1) appears to deteriorate chronic neurodegeneration (Cardona et al., 2006; Garcia et al., 2013), it protects against acute brain injury due to stroke or brain trauma (Dénes et al., 2008; Soriano et al., 2002; Tang et al., 2014; Zanier et al., 2016), whereas time-dependent effects were found concerning cognitive dysfunction after mild brain trauma (Febinger et al., 2015). Similarly, elimination of microglia results in reduced synapse loss and a better cognitive outcome in experimental models of AD or in chronic pain (Sawicki et al., 2019; Spangenberg et al., 2016), whereas it appears to be highly detrimental in different forms of acute brain injury (Bellver-Landete et al., 2019; Fu et al., 2020; Szalay et al., 2016). In these complex models, as well as in human brain diseases, microglia-mediated changes manifest at different temporal and spatial scales, and balancing microglia-mediated phagocytosis of synapses or injured neurons, control of neuronal hyperactivity, extracellular ion balance, and energy metabolism, as well as possible effects on oligodendrocytes, astrocytes, vascular inflammation, or blood-brain barrier injury, could be key to determining overall outcome. As such, parallel microglial actions will collectively shape the resulting pathophysiological consequences via different multicellular and subcellular interactions. To understand this better, the possible structural, molecular, and functional heterogeneity of microglial processes should be investigated. These include the possible presence of a receptor expression/functional gradient along the proximo-distal axis of microglial processes (Umpierre et al., 2020) or functional differences among microglial processes contacting different types of cells in the brain (see Box 1). Functional heterogeneity and autonomy of microglial processes could also explain how a single microglial cell is able to interact with multiple cells in its microenvironment or with different cellular compartments of nearby neurons, which is supported by microdomain-specific calcium signaling in microglia (Umpierre et al., 2020).

Both microglial and neuronal properties (protein expression profiles, cell motility, positions along migratory pathways, etc.) change during development: the landscape of microglial protein expression shows great variability at different maturation levels and across regions, following strict regulation (Masuda et al., 2019; Matcovitch-Natan et al., 2016). Such alterations may underlie the widely observed differences in microglial function and responses with maturation and age. Thus, it will be important to investigate direct and compartment-specific microglia-neuron interactions during neurogenesis, brain development, and aging, among others. Along this line, transcriptomic changes through age and between genders (Guneykaya et al.,

2018) are important to investigate. Future studies should also consider limitations of molecular anatomy approaches (e.g., multicolor immunodetection versus molecular-level resolution), differences between awake and anesthetized mice, species-related features (Miron and Priller, 2020), differences between *in vivo* imaging and measurements in brain slices, or the use of 3D imaging approaches to visualize the rapid movement of microglial processes and process heterogeneity.

To gain deeper insight into the functional aspects of direct microglia-synapse interactions, the molecular mechanisms involved should be extensively studied. For example, it is unclear how complement-mediated or MHC class I-dependent removal of synapses by microglia is regulated by focal release of find-me signals and localized deposition of eat-me signals in opposing cell membranes at the microglia-synapse interface and how these processes are influenced by changes in neuronal activity, mitochondrial function, accumulation of misfolded proteins, or aging. The role of microglia in complement-mediated removal of synapses in different models of AD is already well documented and is important in neuronal loss and dementia (Hong et al., 2016; Luchena et al., 2018). However, the molecular mechanisms that determine the distribution and actions of complement proteins or complement receptors on the synapse-microglia interface and the possible differences among distinct synapse populations need further investigations (i.e., glutamatergic synapses established by different neuronal populations or GABAergic synapse populations based on their postsynaptic subcellular targets: dendritic, perisomatic, and axo-axonic). Many of the open questions concerning microglia-synapse interactions have been critically reviewed in great detail by others (Hong et al., 2016; Shatz, 2009; Whitelaw, 2018; Wilton et al., 2019). In addition, the molecular anatomy and pathways involved in microglia-AIS and microglia-axon interactions, which may determine potentially beneficial and detrimental microglial effects via such direct contact, remain to be established.

Many open questions concern perisomatic microglia-neuron interactions and, specifically, somatic microglial junctions. For example, it would be interesting to know how somatic microglial junctions operate to alter neuronal activity. It is possible that activation of microglial P2Y<sub>12</sub>Rs, which are known to activate THIK-1 K<sup>+</sup> channels in their membrane (Madry et al., 2018a), raises the extracellular [K<sup>+</sup>] locally and thus either stimulates neuronal Na/K pumps, increasing neuronal ATP consumption, or slows neuronal K<sup>+</sup> loss, leading to cell death. Elimination of microglia was found to dysregulate neuronal network activity, alter the induction and propagation of spreading depolarization, and modulate spreading depolarization-associated extracellular potassium levels (Cserép et al., 2020; Varga et al., 2020). Other possibilities include microglia-mediated changes in glutamate spill-over, alterations in neuronal membrane receptor expression/distribution, or regulation of calcium influx via changes in neuronal membranes. Purinergic signaling provides a universal means of cell-cell communication in the brain (Fields, 2011). Because calcium influx through NMDA receptors can play a role in microglia-neuron interactions (Eyo et al., 2014) and ATP-mediated microglial process outgrowth was found to depend on NMDA receptors (Dissing-Olesen et al., 2014), mechanisms of somatic vesicular and non-vesicular ATP release and

exocytosis of other molecules from neurons should be investigated further using selective vesicular release inhibitors, pannexin- or voltage-dependent anion channel inhibitors, and other approaches. Because the important role of ATP and ADP in neuron-microglial signaling had previously been devoted to synaptic ATP release, whereas extrasynaptic release of purinergic metabolites has long been known (Fields, 2011; Stevens and Fields, 2000), the exact cellular sources, sites, and mechanisms of ATP/ADP release should be further investigated. For example, somatic purinergic junctions could be a source of ATP, which activates astrocytes to mediate neurovascular coupling at the capillary level (Attwell and Madry, 2019; Mishra et al., 2016). It also remains unclear why such a long process dwell time (3- to 4-fold over contacts with neuronal dendrites or synapses) is needed for microglia to survey and influence somatic neuronal function (Attwell and Madry, 2019). It is possible that shaping neuronal activity requires prolonged contact between microglia and somatic neuronal membranes.

Communication between microglial processes and neuronal mitochondria appears to be important in understanding how changes in neuronal status are linked with microglial responses under healthy conditions in the aging brain or in brain diseases. Because somatic mitochondria in neurons are implicated in regulating the firing set point (Styr et al., 2019), metabolic control and cell-fate decisions (Bhola and Letai, 2016; Khacho and Slack, 2018; Lorenz and Prigione, 2017), and general signaling (Chandel, 2014), it should be investigated whether microglial regulation of neuronal functions could be achieved through microglial process to neuronal mitochondrion communication. Furthermore, given the central role of mitochondria and mitochondria-related signaling pathways in the somatic junctions, it will be important to study whether mitochondria-microglia interactions could be a more general feature of direct microglial contacts with other cells (see Box 2).

The role of microglia-neuron interactions should be extensively studied in different forms of neurodegeneration, with more emphasis on linking functional observations with precise localization of the key molecules involved using advanced molecular anatomy. Along this line, the molecular anatomy and localization of proapoptotic signals and receptors involved in phagocytosis at the somatic neuron-microglia interface and the precise mechanisms of microglial decision-making need to be comprehensively investigated in future studies. Somatic microglia junctions may be altered in AD, PD, and other forms of chronic neurodegeneration in which excessive release of misfolded proteins such as  $\beta$ -amyloid (A $\beta$ ), p-tau, or  $\alpha$ -synuclein could trigger inflammasome activation (Choi et al., 2020; Masters and O'Neill, 2011; Shi et al., 2015a) and other inflammatory changes in microglia via this interface. Interestingly, hippocampal neurons from 3xTg AD-model mice display loss of function of Kv2.1 channels through increased ROS production, leading to hyperexcitability (Frazzini et al., 2016), and microglia appear to drive neurodegeneration in mouse models of transgenic tauopathy (Shi et al., 2019). Interactions between key proteins that contribute to intracellular sorting of misfolded proteins—such as SORLA, which clears A $\beta$  from neurons and controls its extracellular deposition (Caglayan et al., 2014)—and microglia is also important to study. It remains to be established how

known genetic alterations in different neuropathologies influence microglia-neuron communication. For example in PD, mitochondrial alterations caused by PINK1 and/or Parkin mutations (Ge et al., 2020; Matheoud et al., 2016) may disrupt neuron-microglia communication through somatic junctions. In ALS, misfolded proteins, endoplasmic reticulum stress, and SOD1 mutations could all act on microglia via somatic junctions, and observed disturbances of vesicle trafficking and active-zone proteins (Geloso et al., 2017) might influence microglia-synapse contacts.

In conclusion, recent data strongly suggest that compartment-specific direct interactions among microglia, neurons, and other cells not only enable effective forms of communications but also add another level of complexity to the operation of key mechanisms that should be understood and specifically targeted for better therapeutic perspectives in common neurological diseases.

#### ACKNOWLEDGMENTS

This work was supported by a “Momentum” research grant from the Hungarian Academy of Sciences (LP2016-4/2016 to A.D.) and ERC-CoG 724994 (to A.D.), the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (to C.C.), ÚNKP-20-3-II (to B.P.) and ÚNKP-20-5 (to C.C.) of the New National Excellence Program of the Ministry for Innovation and Technology, Hungary; H2020-ITN-2018-813294-ENTRAIN (to A.D.); and SORLA-FIX 2019-2.1.7-ERANET-2020-00004 from the Ministry for Innovation and Technology, Hungary (to A.D.).

#### DECLARATION OF INTERESTS

The authors declare no competing interests.

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