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Perspective

Microglia: New Roles for the Synaptic Stripper

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Any pathologic event in the brain leads to the activation of microglia, the immunocompetent cells of the central nervous system. In recent decades diverse molecular pathways have been identified by which microglial activation is controlled and by which the activated microglia affects neurons. In the normal brain microglia were considered "resting," but it has recently become evident that they constantly scan the brain environment and contact synapses. Activated microglia can remove damaged cells as well as dysfunctional synapses, a process termed "synaptic stripping." Here we summarize evidence that molecular pathways characterized in pathology are also utilized by microglia in the normal and developing brain to influence synaptic development and connectivity, and therefore should become targets of future research. Microglial dysfunction results in behavioral deficits, indicating that microglia are essential for proper brain function. This defines a new role for microglia beyond being a mere pathologic sensor.

Microglia Are the Cellular Response Element to Pathology

Microglia are an integral part of CNS networks (Figure 1); microglial cells form the innate defensive system of the CNS, and their pathological potential has been extensively investigated (Hanisch and Kettenmann, 2007; Kettenmann et al., 2011). All CNS diseases involve microglia, which typically convert from the resting/surveillant cell type in the normal brain to an activated form specialized to operate within the diseased environment. Microglial activation is classically characterized by two major changes. First, the cell shape transforms from a highly branched and ramified morphology to an amoeboid form, and second, these amoeboid cells become active phagocytes. Recently, it has become apparent that microglial activation is a process more diverse and dynamic than has been considered previously (Hanisch and Kettenmann, 2007). In different pathologies microglia acquire distinct functional states and during the disease progression microglial cells modify and change their activated phenotype. Activated microglia specifically interact with neurons and influence their survival either in a positive or in a negative direction. Microglia can physically contact injured neurons and remove synapses, a process termed *synaptic stripping*; in some instances, entire dendritic trees are eliminated. As part of the activation routine microglial cells release diverse substances such as reactive oxygen species, cytokines or growth factors, which influence the pathological process during the acute and chronic phase as well as during subsequent regeneration.

Microglia Remove Synapses in Pathology: Discovery of Synaptic Stripping

Microglia remove synapses from neuronal cell bodies. This process was first recognized in the facial nerve injury model and termed *synaptic stripping* (Blinzinger and Kreutzberg, 1968). Lesion to the facial nerve that carries axons of motoneurons located in the facial nucleus, triggers microglial activation, manifested by upregulation of cytokines, cell adhesion molecules, extracellular matrix proteins, transcription factors, and proteins of the major histocompatibility complex. Activated microglial cells release neurotrophic factors such as nerve growth factor (NGF), neurotrophin (NT)-4/5, transforming growth factor (TGF)-β1, glial-derived neurotrophic factor (GDNF), fibroblast growth factor (FGF), and interleukin (IL)-3, which affect neuronal survival (Nakajima et al., 2007). Microglia-derived factors also include proinflammatory mediators such as tumor necrosis factor (TNF)-α, IL-6, or nitric oxide (NO), which confer neurotoxicity. Microglial secretory activity can vary depending on the pathologic context (Hanisch and Kettenmann, 2007). In the facial nerve lesion model microglial cells proliferate, migrate to and interact with motoneurons ultimately removing synaptic input to these cells (Moran and Graeber, 2004). In the rat facial nucleus the response is exclusively restricted to intrinsic microglia and does not involve invading blood cells such as macrophages or lymphocytes. The phenomenon of synaptic stripping represents a generic microglial response, which can also be activated in other brain regions (e.g., in the cortex [Trapp et al., 2007]). In certain pathologies, for example in prion disease, activation of microglia proceeds without activation of synaptic stripping (Siskova et al., 2009; Perry and O'Connor, 2010).

There are many factors, by which microglia sense neuronal injury, including chemokines or small molecules like ATP. Conceptually, two types of signals control microglial behavior in response to injury: "*find-me*" signals attract microglial cells to the damaged site, whereas "*eat-me*" signals allow microglia to identify the target and trigger phagocytosis. Another classification distinguishes between "on signaling," which includes factors newly appearing in the pathologic context or are upregulated and "off signaling," which includes factors that disappear or are downregulated in pathology (Biber et al., 2007). Observations from vagal motoneurons additionally suggest that a

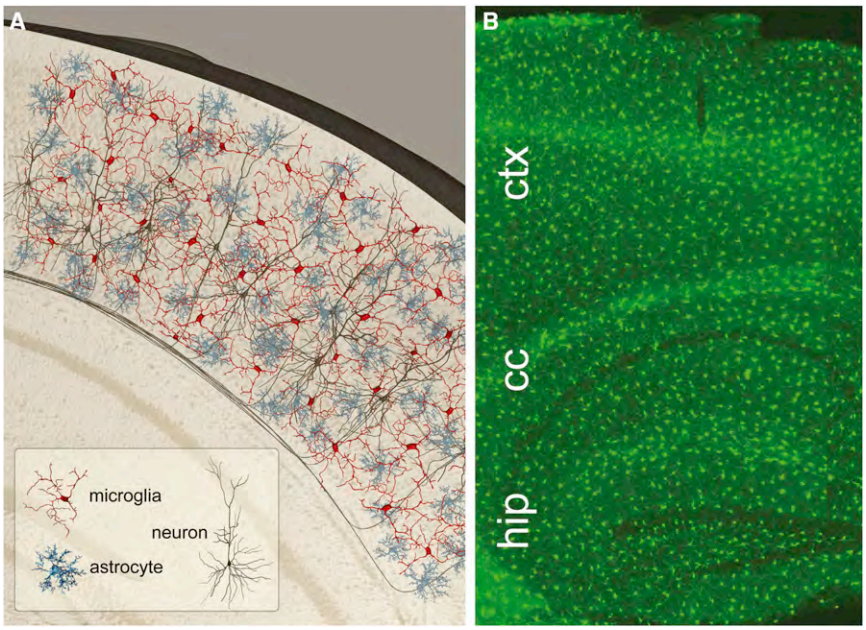

Figure 1. Uniform Distribution of Microglia in the Central Nervous System

(A) Throughout the central nervous system microglia (red) surveys neuronal networks (black) and astroglial syncytia (blue). Both microglia and astrocytes uniformly divide the gray matter through a process called tiling in which individual microglial cells and astrocytes only minimally overlap in the three-dimensional space. However, processes of one cell type can strongly overlap with territories of the other cell type. While astrocytes are part of rather stable structure-functional elements known as neurovascular units, microglial processes constantly scan through their territorial domains and establish frequent transient contacts with neighboring neurons and astrocytes.

(B) The panel shows a laser-scanning micrograph taken from an adult TgH(CX3CR1-EGFP) mouse brain in which microglia is labeled by expression of EGFP. Note the uniform cellular distribution within and across different brain regions such as cortex (ctx), corpus callosum (cc), and hippocampus (hip).

decrease in synaptic activity precedes (and may potentially initiate) synaptic stripping (Yamada et al., 2008).

Microglial Cells Can Sense Neuronal Activity

Studies in cell culture indicated that microglial cells express a variety of receptors for neurotransmitters, neuropeptides, and neuromodulators and thus have the capacity to sense neuronal activity (Pocock and Kettenmann, 2007). For example, cultured microglia express adrenergic receptors, metabotropic and ionotropic glutamate and γ-aminobutyric acid (GABA) receptors, dopamine receptors, bradykinin receptors, and several types of purinoceptors; for some of these receptors functional expression was corroborated by experiments in situ, as it has been shown for serotonin, purinergic, endothelin-1, histamine, substance P, and $GABA_B$ receptors (Kettenmann et al., 2011; Krabbe et al., 2012; Seifert et al., 2011). So far, the expression of these receptors in microglia has only been studied in acute brain slices where the microglial activation process is already initiated. It is conceivable, however, that microglial cells in the unperturbed brain already express many (if not all) of such receptors. The cell culture studies show that stimulation of receptors to neurotransmitters, neuropeptides, and neuromodulators affect microglial function instigating cytokine release, movement of processes in response to injury or phagocytosis (Kettenmann et al., 2011). For the inhibitory transmitter GABA an anti-inflammatory action on microglia has been suggested (Lee et al., 2011). Partial activation of both $GABA_A$ and $GABA_B$ receptors renders microglia less responsive to inflammatory stimuli such as lipopolysaccharide (LPS) and interferon-γ (Lee et al., 2011). Although a diversity of transmitter receptors has been described on microglia, so far we lack experimental proof that these receptors are activated during synaptic transmission. Studies taking advantage of microglia-selective gene knockout models or microglia-specific functional rescue experiments in a constitutive knockout background may resolve this issue.

"Resting" Microglia Are Active Cellular Elements

Migration of myeloid cells into the nervous tissue occurred early in the evolution of the nervous system, and microglia-like elements responsive to pathological insults are present in ganglia of annelids (e.g., leech) and in mollusks. In vertebrates, the development of the CNS is associated with a significant increase in the density of parenchymal microglia, which account for ~10% of the total brain cell population in mammals (Lawson et al., 1990; Mittelbronn et al., 2001). After entering the brain, myeloid cells undergo remarkable remodeling that change their structural appearance and physiology. Microglia acquire a specific morphology (a small cell body endowed with several extra-thin and highly motile processes) a state, which was termed "resting microglia." By experiencing this profound metamorphosis, microglia adapts to the neural environment, and, as we will discuss later, become an integral part of the neural circuitry (see also Figure 1), essential for CNS development, (re)modeling, and physiological plasticity. It is also important to remember that microglial cells display significant morphological heterogeneity between brain regions (Olah et al., 2011), whereas their functional heterogeneity awaits detailed investigation.

A paradigm shift from recognizing microglia only as "resting" cells waiting for pathology, occurred when microglial process motility was directly observed in the living mouse by in vivo two-photon laser-scanning microscopy. In these experiments microglial cells were imaged in the brain through either a cranial window or a thinned scull, thus keeping CNS tissue undisturbed (Davalos et al., 2005; Nimmerjahn et al., 2005). These observations revealed that microglial processes are arguably the fastest moving cellular structures (with more than 1 to 3 μm/min

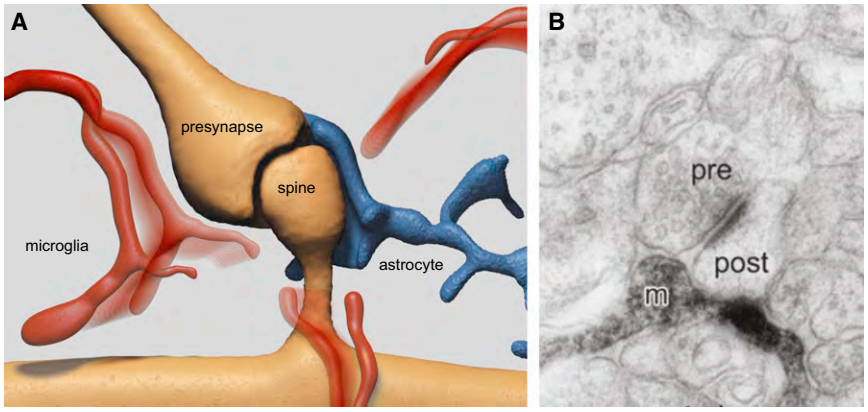

Figure 2. Dynamic Interaction of Microglial Processes with the Tripartite Synapse
(A) Microglial processes (red) dynamically contact the cellular compartments of the tripartite synapse: pre- and postsynaptic neuronal terminals (in brown) as well as the enwrapping perisynaptic astroglial process (in blue).
(B) The electron micrograph (EM) specifically shows a microglial process (m) contacting both the pre- and postsynaptic compartment. The EM image is modified from Wake et al. (2009).

process extensions and retractions) in the intact healthy brain, thereby voiding the concept of microglia as a resting element. In a retinal slice preparation Fontainhas et al. (2011) provided evidence that neural activity mediated by GABAergic and glutamatergic mechanism regulate this movement of processes. They conclude, however, that the motility control of microglial processes is mediated through a purinergic signaling cascade. In the in vivo experiments by Davalos et al. (2005) it was evident that lowering extracellular ATP concentration by the ATP-hydrolyzing enzyme apyrase results in reduced process movements, whereas artificially created ATP gradients stimulate their motility. The metabotropic $P2Y_{12}$ purinoceptors were found to be responsible for that type of movement control (Haynes et al., 2006).

Microglial Cells Contact Synapses in the Normal Brain

In the absence of pathological insults the highly dynamic surveillant motility of microglia is specifically targeted to synaptic structures (Figure 2). In the somatosensory and visual cortex two-photon microscopy revealed that the microglial processes make brief, repetitive contacts with synapses at a frequency of about once per hour. These interactions were visualized by in vivo two-photon imaging in the Iba-1-EGFP/Thy-1-GFP double-transgenic mice in which microglial cells and neuronal structures can be simultaneously visualized. Wake et al. (2009) reported that the microglial processes appear in a close proximity to presynaptic boutons, where they remain for about 5 min and then retract. These structural interactions are activity-dependent, as their frequency was reduced by decreased neuronal activity following either binocular eye enucleation, injection of tetrodotoxin into both retinae or a reduction of body temperature (Wake et al., 2009). By combining two-photon in vivo imaging with immunohistochemistry and three-dimensional reconstructions obtained from serial section electron micrographs (Figure 2B), a rather specific apposition of microglial processes to pre- as well as postsynaptic compartments was found in the visual cortex of juvenile mice (Tremblay et al., 2010). Process extrusions were typically associated with small and transiently growing dendritic spines. Again, neuronal activity modulated microglial behavior: light deprivation reduced the motility of microglial processes and increased their association with larger dendritic spines. Re-exposure to light reversed these behaviors and microglial processes enwrapped synapses more extensively (Tremblay et al., 2010). Thus microglial cells respond to altered sensory experience and it remains an open question whether the interactions play any role in experience-dependent modification or elimination of synapses, in the developing as well as in the adult CNS. Molecular cues that attract microglial processes to the synapses remain largely unknown, although ATP and purinoceptors are good potential candidates.

Microglia Release Substances that Can Affect Neurons

Conceptually, microglial cells can affect neural networks either through removal of cellular and subcellular elements (by phagocytosis) or through secreting various factors with transmitter, trophic or neuroprotective properties. The variety of neuroactive agents that microglial cells can secrete has been mainly studied in cell culture, an environment that triggers transformation of microglial cells into an activated state. Thus, these factors are considered to be pathological signals, which include several types of cytokines (e.g., TNF-α or ligands for receptors such as CCR1, 3, 5, and 7 and CXCR1 or 3), trophic factors like brain derived neurotrophic factor (BDNF), the gaseous transmitter NO or neurotransmitters (ATP and glutamate) (for review, see Kettenmann et al. [2011]). Some of these substances were reported to rapidly modulate neuronal function by changing excitability and synaptic strength. Meanwhile, several factors known from pathology, can mediate the interactions of microglia with synapses in the healthy brain.

Recent findings indicate that some of these "pathological" factors mediate neuron-microglia crosstalk in the developing or uninjured adult brain. Fraktalkine and complement receptors can mediate synaptic pruning, whereas TNF-α affects synaptic scaling. These processes represent only the beginning of new discoveries of factors that mediate neuron-microglia crosstalk in the normal brain. The fact that most of microglia-derived factors are considered to be pathological may reflect global upregulation in response to pathology. In a physiological context the upregulation of some factors may be structurally confined to distinct sites within the microglial cell (at sites where they interact with neuronal structures) or confined to a small population of microglia that is engaged in a defined interaction with neurons. This could be the reason why some of these neuron-microglia signaling factors have so far remained undetected, and their discovery awaits future, more sensitive detection methods.

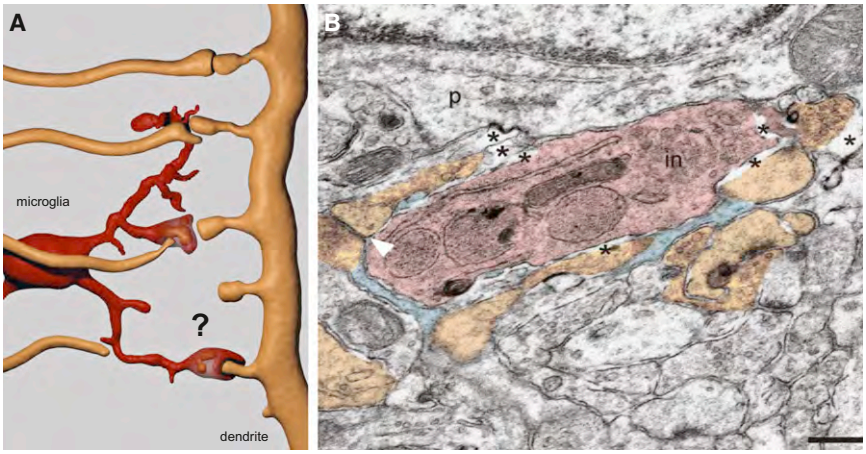

Figure 3. Synaptic Pruning by Microglial Processes

(A) The stability and maintenance of presynaptic terminals and postsynaptic spines is determined by microglia in a three-step process called synaptic pruning composed of contact, engulfment, and phagocytosis of presynaptic terminals. Whether dendritic spines are similarly removed by microglia is still unclear.

(B) The electron microphotograph shows ultrastructural interactions between microglia (red) and synapses (brown) in the mouse visual cortex. In the thickened microglial process inclusions (in) can be recognized (modified from Tremblay et al. [2010]). The asterisks indicate extended extracellular space adjacent to the microglia. Thin processes of perisynaptic astrocytes are shown in light blue. The arrowhead points toward a synaptic cleft. Scale bar = 250 nm.

Microglia Mediate Synaptogenesis and Synaptic Pruning during Development

The first wave of microglial ancestors, the primitive myeloid progenitors originating from the extra-embryonic yolk sac, enter the nervous tissue very early in embryonic development, being in essence the first glial cells in existence (as both astrogliogenesis and oligodendrogliogenesis occur later in a perinatal period) (Ginhoux et al., 2010). This initial migration coincides with the first wave of embryonic synaptogenesis (which occurs, in rodents, around embryonic day 14–15) that proceeds in the absence of astrocytes (which assist and are indispensable for postnatal formation of synapses). In this phase microglia can assist and even promote early synaptogenesis through secretion of growth factors. During later, pre- and postnatal development, microglial processes actively engulf synaptic structures and exert a major role in controlling the number of synapses through synaptic pruning (Figure 3). The chemokine fractalkine plays a role in chronic pain, inflammation, and Alzheimer's disease and is released by neurons and endothelial cells (Clark and Malcangio, 2012; Ransohoff et al., 2007). In the brain, fractalkine receptors are expressed specifically by microglia and fractalkine receptor-driven EGFP expression has become a reliable marker for identifying microglial cells (Jung et al., 2000). In mice deficient for this receptor, there is a transient increase in the spine density during development, suggesting an underlying deficit in synaptic pruning. Depletion of fractalkine receptor also increased the frequency of miniature excitatory postsynaptic currents recorded in the hippocampus. This demonstrates that deficits in microglia function result in synaptic changes (Paolicelli et al., 2011). It could be speculated that a microglial dysfunction could be responsible for developmental disorders, which result in autistic conditions and psychiatric diseases such as schizophrenia. In disease models, fractalkine receptor deficiency conveys neurotoxicity. This was observed following peripheral lipopolysaccharide injections, in a toxic model of Parkinson disease and a transgenic model of amyotrophic lateral sclerosis (Cardona et al., 2006). Importantly the physiological and pathological phagocytosis show morphological specificity: the phagocytosis of synaptic material or apoptotic cells is performed without affecting the ramified microglial phenotype, by microglial processes or "en passant" branches forming "ball-and-chain" structures (Sierra et al., 2010). The phagocytic activity of ramified microglia can be recorded in acute slices and seems to represent a constitutive behavior. Amoeboid microglia in situ also extend processes that identify and phagocyte the pathogenic material, although these processes have a velate appearance quite different from processes in ramified microglia. Despite this structural distinction the underlying molecular mechanisms may be similar. Incidentally it is differently regulated in early postnatal and adult microglia since only in early postnatal microglia the phagocytic activity is controlled by serotonin (Krabbe et al., 2012). The molecular cues regulating the synaptic pruning and elimination during visual experience still have to be identified.

Receptors of the Complement Cascade Play a Role in the Removal of Synapses by Microglia

Members of the complement cascade like C5a or C3a are sensed by cultured microglial cells and trigger an activated phenotype (Ischner et al., 1996). The C1q complex also acts as an activation signal for cultured microglial cells because it shifts these cells toward a proinflammatory phenotype manifested by the release of IL-6, TNF-α, and NO as well as an induction of an oxidative burst similar to the proinflammatory response of microglia to LPS (Färber et al., 2009). Recently, C1q has been identified as an important factor for controlling synaptic pruning in the developing nervous system. During development, C1q is expressed by neurons and localized to synapses throughout the postnatal CNS and retina (Stevens et al., 2007). The authors speculate that C1q serves as a tag for synapses that need to be eliminated. Microglial cells engulf presynaptic inputs during peak retinogeniculate pruning around postnatal day 5, a time point when the phagocytic activity of microglia is high. The response is mediated by the complement receptor 3 (CR3, composed of CD11b and CD18 integrins) because the phagocytic activity was lower in animals deficient for that receptor, and genetic deletion resulted in deficits in synaptic connectivity (Schafer et al., 2012).

Microglia Regulate Synaptic Plasticity

Several studies indicate that microglial cells can influence synaptic plasticity. Examples range from modulation of the NMDA

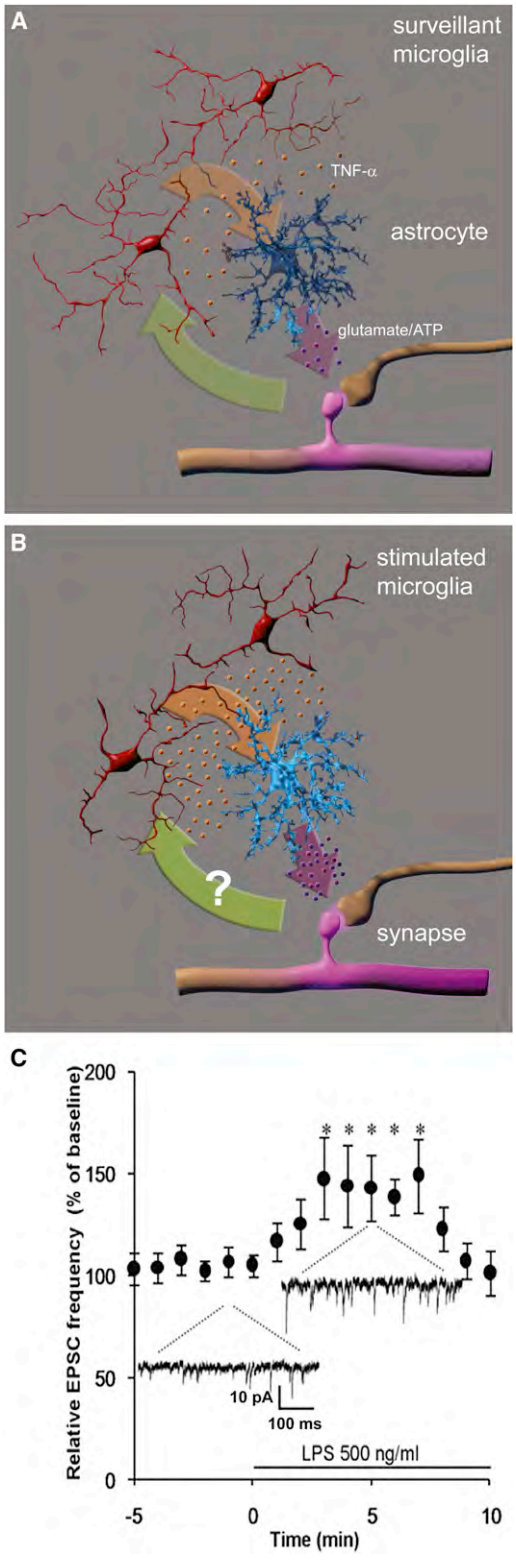

Figure 4. Microglial Regulation of Synaptic Plasticity in a Multicellular Network

Microglial cells constitutively release different cytokines. This release is highly sensitive to environmental condition and can be augmented over several orders of magnitude. Enhanced microglial TNF-α release has been shown to stimulate the release of glutamate and/or ATP from adjacent astrocytes. Subsequently, these compounds can act pre- and postsynaptically to strengthen synaptic transmission in a process called synaptic scaling.

(A) Baseline level of microglia function during network activity.

(B) Stimulation of microglia results in astroglial triggers of enhanced synaptic activity. The mechanisms, however, how microglia perceive alterations of synaptic activity have still to be uncovered.

(C) Microglial stimulation by LPS increases the firing rate of AMPAergic synapses in acute hippocampal slices of the mouse brain. Insets show representative traces before and after LPS application. The EPSP frequency has been recorded from voltage-clamped pyramidal neurons. Modified from Pascual et al. (2012).

receptor glycine binding site by microglia (Hayashi et al., 2006), signaling by fractalkine and its receptor (Paolicelli et al., 2011; Rogers et al., 2011), modulation of Cl^- gradient in neurons through microglial BDNF release (Coull et al., 2005) and purinergic signaling (Tsuda et al., 2010). The best documented role of microglia in controlling neural network functions, however, comes from the analysis of TNF-α effects on synaptic connectivity. TNF-α is a proinflammatory cytokine, which is released by microglial cells. Rise in TNF-α is a hallmark of acute and chronic neuroinflammation as well as various neuropathological developments including ischemic stroke, Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis, and multiple sclerosis (McCoy and Tansey, 2008). Under physiological conditions, the low levels of TNF-α (acting at about 100 picomolar concentrations) is a potent effector of synaptic scaling (Pascual et al., 2012; Stellwagen and Malenka, 2006), a uniform adjustment in the strength of all synapses formed on a given neuron in response to prolonged changes in the electrical activity. Incubating hippocampal slices with TNF-α increased the ratio of AMPAR- to NMDAR-mediated synaptic currents without affecting long-term potentiation (LTP) or long-term depression (LTD). By using cocultures of neurons and glia from wild-type or TNF-α knockout mice, the source of TNF-α was attributed to glia. Thus, by modulating TNF-α levels, neuroglia actively participate in the homeostatic activity-dependent regulation of synaptic connectivity (Stellwagen and Malenka, 2006).

More recent data (Pascual et al., 2012) demonstrate that the TNF-α is exclusively generated by microglia, and not by the astrocytes. The scaling effect of TNF-α is mediated by activation of astroglial TNF receptor I (TNFR I) (Bezzi et al., 2001; Domercq et al., 2006). Microglial TNF-α and microglial ATP act in concert to trigger adjacent astrocytes to release ATP. Astroglial ATP subsequently amplifies the microglial signal and promotes astroglial release of glutamate that directly affects synaptic transmission through presynaptic metabotropic glutamate receptors (Figure 4 and Pascual et al., 2012). These experiments performed in the hippocampus provide important insights into the complexity of neural circuits. Similar regulatory mechanisms probably exist in the developing brain, for example in the developing visual cortex. After monocular visual deprivation, the reduction in response of binocular neurons to one eye is matched by a corresponding increase to the other. In mice deficient in TNF-α the increase in response to the open eye is absent, thereby suggesting an experience- and TNF-α-dependent mechanism (Kaneko et al., 2008). A similarly complex interaction of microglial ATP release, purinergic receptor activation,

Ca^{2+} signaling, and subsequent glutamate release has been also described in lower vertebrates namely in zebrafish, though during the onset of an acute injury (Sieger et al., 2012).

Microglia Regulate Adult Neurogenesis

In the adult rodent brain, two neurogenic niches localized in the subventricular zone and the dentate gyrus of the hippocampus produce new neurons. There are conflicting findings as to the role of microglia in regulating neurogenesis. An attenuating influence of microglia is inferred from the finding that inflammation impairs the ability of hippocampal neural stem cells to generate new neurons and this ability can be restored by anti-inflammatory drugs such as indomethacin (Monje et al., 2003). In contrast, the in vitro data indicate that microglial cells promote neurogenesis. Supernatant from microglial cultures endorses the ability of stem cells to self-renew and to form multipotent neurospheres (Walton et al., 2006). Hippocampal neurogenesis induced by an exposure to the enriched environment was associated with the recruitment of T cells and the activation of microglia (Ziv et al., 2006), which further supports the facilitating role of microglia. In the adult hippocampus microglia contributes to the regulation of network activity by controlling the integration of newly born neurons into the existing circuits and elimination of supranumerous, apoptotic neurons (Sierra et al., 2010).

Microglial Dysfunction Affects Behavior

Proper microglial function is a prerequisite for appropriate brain function and constitutive activity of microglial cells seems to be important for maintenance of neuronal circuitry and control of behavior. There are two intriguing findings illustrating that selective dysfunction of microglia leads to abnormal behavior or neural dysfunction. The first example is a mouse model of Rett syndrome, a form of autistic disorder characterized by impaired synaptogenesis resulting in severe disturbances of motor, language, and cognitive functions. These deficits correlate with decreased size of neurons, reduced dendritic branching, and reduced number of spines. The disease is linked to mutations in the gene for methyl CpG binding protein 2 (MECP2), a transcriptional repressor probably expressed in all cell types of the brain. Mice with deletion of MECP2 exhibit impaired locomotor function and a reduced life span. Microglia deficient in MECP2 show a strong reduction in phagocytic activity. Interestingly, the phenotype of MECP2 knockout mice can be partially rescued by selective expression of MECP2 in cells of the monocytic lineage, including microglia. Thus, constitutive microglial phagocytic activity appears to be essential for the development or maintenance of neuronal circuitry (Derecki et al., 2012).

Another brain dysfunction related to microglia is the pathological grooming behavior in Hoxb8-deficient mice. These mice are used as a model for a compulsive hair pulling human disorder also known as trichotillomania. In the mouse model, the behavioral phenotype can be rescued by transplanting wild-type bone marrow cells into these mutant mice. It is assumed that the bone marrow derived monocytes populate the brain and transform into cells with microglial properties. The rescue experiment by bone-marrow grafting in adult mice suggest that this is not a developmental defect, but may reflect dysfunction of microglia in the adult animals (Chen et al., 2010).

Microglial Dystrophy Contributes to Brain Aging

There is increasing evidence for microglial senescence that may contribute to overall brain aging and age-associated cognitive decline. Advanced age affects microglial phenotype by changing their morphology (deramification, increase of the cell body, fragmentation of the cytoplasm, formation of cytoplasmic spheroids, etc.). These changes in microglial morphology are apparently global and occur throughout the nervous system (Lopes et al., 2008; Streit et al., 2004). This has also been thoroughly analyzed in the auditory and visual cortex indicating that microglial density increased resulting in smaller territories of individual cells. Moreover aged microglia have a more variable cell body and process morphology (Tremblay et al., 2012). These dystrophic microglial cells also display functional abnormalities manifested by slower process motility and impaired reactions to local injury (Damani et al., 2011). The dystrophic microglia is arguably less capable in protecting CNS homeostasis and may promote neurodegenerative processes. In the absence of pathology dystrophic microglia can contribute to overall weakening of synaptic connectivity and plasticity, which generally underlie age-dependent cognitive decline. These age-dependent alterations further highlight the importance of microglia for normal brain function.

Microglia Modulate Neuronal Networks: Further Lessons from Pathology

As discussed above there are many potential factors known from pathology, which can mediate neuron-microglia crosstalk (Figure 5). We also speculate that many factors have remained undetected since they may be restricted to small subpopulations of microglia or even small compartment(s) of the microglial cell. In astrocytes for example, signaling often appears in microdomains restricted to small areas within processes (Grosche et al., 1999). Below, we present three examples, so far studied only in pathology, which show how microglia can affect neuronal networks.

Is CCL21/CXCR3 Interaction Relevant for Removal of Dendrites by Microglia?

Neurons damaged by glutamate excitotoxicity rapidly express and secrete the chemokine CCL21; the latter is packaged into vesicles, transported to presynaptic structures, and released through exocytosis (de Jong et al., 2005). Microglial cells express CXCR3 receptors specific for CCL21. Stimulation of microglia with CCL21 triggers chemotaxis and increases migratory activity (Biber et al., 2001). This neuron-microglia crosstalk has been studied in the entorhinal cortex lesion model. When the projections from the entorhinal cortex to the dentate gyrus of the hippocampus are severed, the input parvalbumin-positive interneurons undergo degeneration of their dendritic tree, the latter being located in the molecular layer. Microglial cells are attracted to that site. When, however, the CXCR3 receptor is genetically deleted, microglial cells are not recruited anymore and the dendritic trees of the interneurons remain intact (Rappert et al., 2004). This finding indicates that removal of synaptic structures is an active process that involves microglia and a chemokine signaling cascade.

When Do Microglial Cells Contribute to Glutamate Clearance?

One important function of astrocytes is the removal of excess glutamate by the activity of glutamate transporters. It is assumed

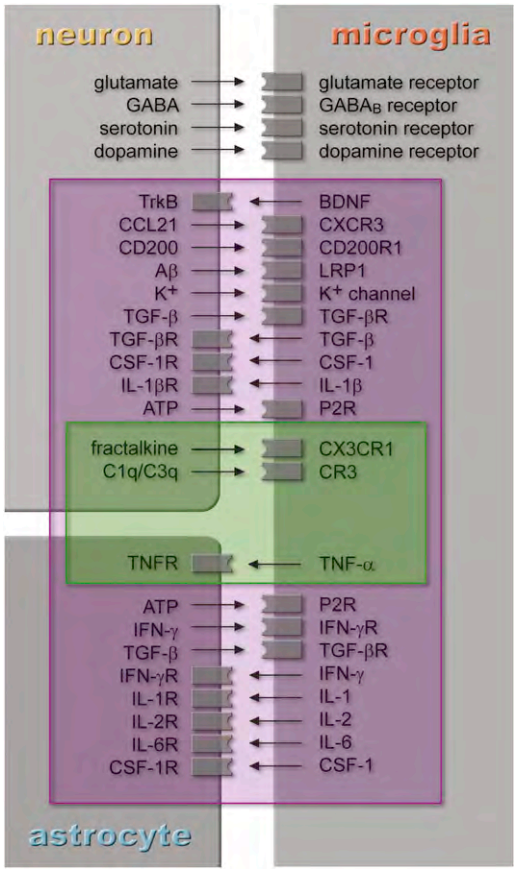

Figure 5. Neuron-Microglia and Astrocyte-Microglia Signaling Pathways in Pathology and Physiology

This scheme provides a highly simplified list of signaling pathways that have been described in vitro or in vivo. The magenta box highlights many of those that have been identified in pathology only. The green box, in contrast, indicates signaling pairs with demonstrated functions at physiological conditions. Most of the transmitter receptor signaling, however, has not yet been associated with a disease or confirmed to be present in physiology (for details see Kettenmann et al. [2011] and Kettenmann and Ransom [2012]).

that the majority of glutamate that is released during synaptic activity is transported into astrocytes. This ensures low levels of extracellular glutamate and is a prerequisite for maintaining transmitter homeostasis (Danbolt, 2001). Under pathologic conditions, microglial cells upregulate the glutamate transporter GLT-1 (EAAT-2), which is claimed to play a role in glutamate clearance (van Landeghem et al., 2001). This was first described in the facial nerve injury model. The induced expression of glutamate transporter suggests a neuroprotective role of microglia against glutamate excitotoxicity following nerve axotomy (López-Redondo et al., 2000).

Under What Conditions Can Microglial BDNF Regulate GABAergic Signaling?

Microglial cells are capable to modulate synaptic transmission by secreting factors that affect synaptic responses. In an animal model of neuropathic pain, activation of $P2X_4$ receptors on spinal cord microglia stimulates release of BDNF (Trang et al., 2011; Tsuda et al., 2010). BDNF, in turn, causes a depolarizing shift in the chloride reversal potential in spinal lamina I neurons thus reversing the direction of $GABA_A$ receptor mediating currents. As a result, microglia-derived BDNF converts inhibitory GABA/glycine responses into excitatory ones thereby affecting the excitability of the neuronal network. Interfering with purinergic signaling or attenuating microglial BDNF release reduced symptoms of neuropathic pain. BDNF from spinal microglia is therefore a critical microglia-neuron signaling molecule that gates aberrant nociceptive processing in the spinal cord (Trang et al., 2011; Tsuda et al., 2010). It is tempting to speculate that similar mechanisms also exist in the healthy brain, e.g., during plastic events that occur in learning and memory formation.

Conclusion

Substantial evidence has been accumulated that show the wide potential of microglia to sense various activities of their adjacent cellular neighbors, of neurons as well as astrocytes or microglia themselves. Processes of astrocytes as well as microglia are in close proximity to synapses. While astroglial contacts are considered to be a more permanent component of the "tripartite synapse," microglial processes are much more dynamic and display only transient interactions.

In particular, microglia fulfills their function in shaping the brain during development and plasticity. In addition, its strength as an environmental sensor and powerful provider of cytokines, growth factors or transmitters establishes microglia as a potent and far-reaching regulator of the extended neuron-glia network. This further advances the doctrine regarding the brain as a multicellular circuitry in which all cell types are acting in concert to achieve the most effective information processing and decision making.

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