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Review
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Sublime Microglia: Expanding Roles for the Guardians of the CNS

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Recent findings challenge the concept that microglia solely function in disease states in the central nervous system (CNS). Rather than simply reacting to CNS injury, infection, or pathology, emerging lines of evidence indicate that microglia sculpt the structure of the CNS, refine neuronal circuitry and network connectivity, and contribute to plasticity. These physiological functions of microglia in the normal CNS begin during development and persist into maturity. Here, we develop a conceptual framework for functions of microglia beyond neuroinflammation and discuss the rich repertoire of signaling and communication motifs in microglia that are critical both in pathology and for the normal physiology of the CNS.

Introduction

The pervasive view of microglia is that their singular function is as inflammatory cellular mediators in diseases of the CNS. This view was seemingly cemented with the discovery that, within the lattice-like pattern of adjacent, nonoverlapping territories, microglia respond within minutes to even highly restricted areas of damage as small as a single neuronal process (Davalos et al., 2005; Nimmerjahn et al., 2005). Moreover, microglia adopt stimulus-dependent phenotypes in response to more widespread injury, infection, or disease. The response repertoire of microglia includes cytoskeletal rearrangements leading to morphological changes, stereotypic transcriptional alterations, and proliferation. Thus, microglia keep vigilant for disruptions to the status quo in the CNS, responding to specific threats in highly programmed ways and calling in reinforcements as needed.

But most of the time the CNS is not under imminent threat or attack. What then are microglia doing, if anything, in the normal, healthy CNS? With increasingly sophisticated tools, it has been possible to gain insights into this question probing not only the functions of microglia in injury and disease, but also their functions under healthy conditions. The concept that microglia may participate in healthy CNS function was blown wide open by the discovery that microglia processes are highly active, continuously scanning the environment (Davalos et al., 2005; Nimmerjahn et al., 2005). Subsequent results have been astonishing, revealing unexpected roles for microglia in normal development, connectivity, and plasticity in the CNS. A common theme across the physiological roles for microglia is that these cells can be seen as instructive, unlike during or following injury, infection, or disease, during which the microglia are viewed as reactive (Schafer et al., 2013; Tremblay et al., 2011; Wake et al., 2013). Thus, microglia are not restricted to responding when things go wrong but are active players in shaping activity in the healthy CNS.

More Than Just Inflammatory Cells

Microglia constitute ~10% of the total cells in the adult CNS, though there is considerable heterogeneity in microglia density among CNS regions (Aguzzi et al., 2013; Kettenmann et al., 2013; Tremblay et al., 2011). Microglia are often considered to be macrophages of the CNS, but a series of recent findings in the mouse has established that microglia are a unique cell population distinct from macrophages. Fate mapping has revealed that adult microglia are derived from precursors that leave the yolk sac on E8.5–E9.0, entering the neural tube via the primitive bloodstream (Ginhoux et al., 2010;Greter and Merad, 2013) (Figure 1). The erythromyeloid progenitors that give rise to migrating cells have been identified as cells with low expression of CD45 and high expression of c-kit (Kierdorf et al., 2013). As the progenitor cells journey to the CNS, they gain lineage-specific gene expression and ultimately differentiate into mature microglia. The yolk-sac-derived microglia remain throughout life, with the population being maintained by self-renewal in the healthy CNS with little contribution from bone-marrow macrophages (Ajami et al., 2007). Microglia development is independent of the transcription factor Myb and of colony-stimulating factor 1 (CSF-1) (Schulz et al., 2012), both of which are essential for bone-marrow-derived macrophages (Hashimoto et al., 2013; Yona et al., 2013). By contrast, microglia depend upon the CSF-1 receptor (Erblich et al., 2011) through its alternate ligand IL-34 (Greter et al., 2012), which is principally produced by neurons (Wang et al., 2012). PU.1-dependent and IRF8-dependent pathways are also critical for microglia (Kierdorf et al., 2013). The persistence of microglia in the CNS, but not the initial seeding from yolk sac, depends upon TGFβ-1 in

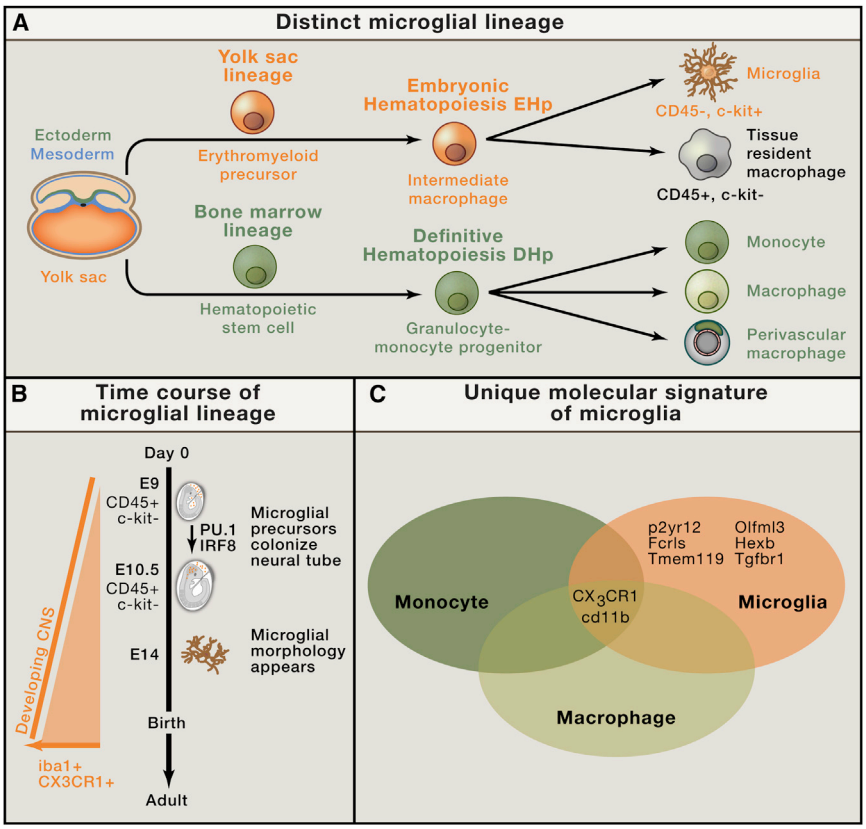

Figure 1. Microglia Have a Distinct Lineage and Molecular Signature

(A) The genesis and progression of yolk-sac-derived cell lineages are illustrated. Microglial are derived from primitive erythromyeloid progenitors in the yolk sac (embryonic hematopoiesis, indicated in yellow), distinct from the definitive hematopoiesis (shown in green) from which the majority of macrophages are derived.

(B) An illustration of the time course of microglial derivation and embryonic colonization as revealed by fate-mapping experiments. Microglia originate from PU.1-dependent precursors in the yolk sac that proliferate and invade the neuroectoderm-derived developing CNS, as indicated by an increase in the markers CX_3CR1 and iba1 (shown as orange ramp).

(C) The diagram illustrates the overlap in gene expression between microglia, macrophages, and monocytes. Two of the most widely used "markers," CX_3CR1 and cd11b, are common to all three. Although there is considerable molecular similarity between tissue-resident macrophages and microglia, a subset of microglial-specific genes have been identified, and the six most prominent of these genes are shown (see Butovsky et al. [2014]).

the CNS (Koeglsperger et al., 2013), whereas this cytokine is not required for bone-marrow-derived macrophages (Hashimoto et al., 2013).

Transcriptome analysis has revealed that microglia have a distinctive signature as compared with circulating macrophages but are closely related to other tissue-resident macrophages developing from primitive yolk sac (Butovsky et al., 2014; Hickman et al., 2013). A host of microglia-specific transcripts and proteins, as well as microRNA, have been identified, characterizing the gene network expressed by microglia in the adult brain under normal conditions. Moreover, the stimulus-induced transcriptional plasticity of microglia does not correspond to the M1 or M2 plasticity observed with macrophages (Mills, 2012) and is distinct from the resting state transcriptome, which has been termed M0 (Koeglsperger et al., 2013). Thus, although microglia and macrophages express a number of common proteins, some of which are very well known such as CD11b (also referred to as integrin α_M, ITGAM), overall they are distinct and readily distinguishable cell populations. Even when bone-marrow-derived macrophages infiltrate the CNS, for example, during experimental autoimmune encephalomyelitis (EAE), these cells retain their transcriptional signature and do not adopt that of endogenous microglia (Ajami et al., 2007; Koeglsperger et al., 2013; Saederup et al., 2010). The dramatic transcriptome differences between microglia and macrophages and other immune cells highlight that microglia are not classical immune cells. A rapidly growing body of evidence is increasingly demonstrating that microglia have a variety of nonphlogistic functions in the normal, healthy CNS. Thus, one may question whether microglia are principally inflammatory cells. As inflammation has emerged to be just one aspect of their function, microglia are rightly considered dual-functioning cells that are critical in the normal as well as in the diseased CNS.

The Microglia "Surveillance and Rapid Response" State in the Healthy CNS

In the normal adult CNS, microglia have a complex morphology with highly ramified processes extending from a compact cell body that continuously scan the environment transiently contacting synapses as well extrasynaptic regions. The microglial processes directly appose not only presynaptic terminals and dendritic spines, but also perisynaptic astrocytic clefts (Tremblay et al., 2010). Here, microglia are well positioned to monitor neuronal firing activity and synaptic function, as they express receptors for numerous types of neurotransmitters and mediators (Kettenmann et al., 2011; Koizumi et al., 2013). In response to neuronal activity, microglia steer their processes toward active synapses (Figure 2A), which facilitates contact with highly active neurons. Using live imaging in the mouse, microglia processes have been found to not only passively monitor neuronal activity, but also actively respond (Tremblay et al., 2010; Wake et al., 2009). In zebrafish, microglial processes have been observed to enwrap the soma of highly active neurons (Figure 2A) and subsequently the firing of the targeted neuron decreases (Li et al., 2012). Thus, microglia are not "resting" but are highly active in a "surveillance and rapid response" state in which they monitor and control the activity of neurons even in the healthy CNS.

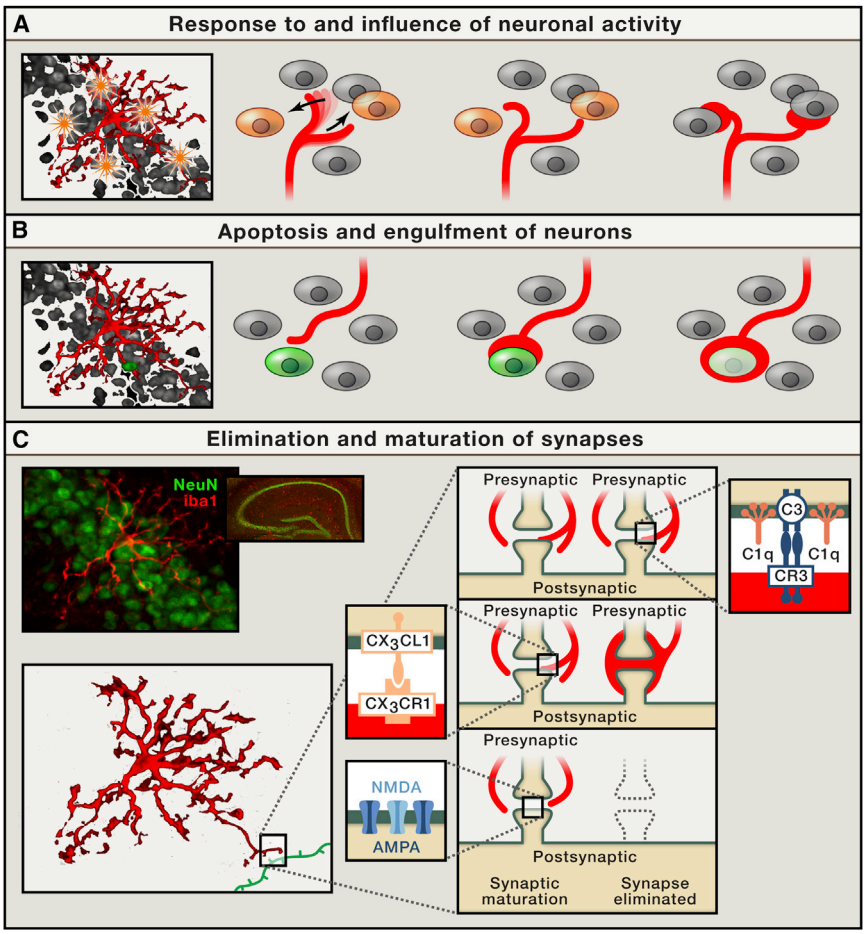

Figure 2. Microglia Control Neuronal Activity, Programmed Cell Death, and Synapse Connectivity

(A) Microglia can respond to and, in turn, influence neuronal activity. The low power image on the left illustrates a microglia (red) with interspersed neurons of high (orange) or low (gray) activity. The high-power cartoon images illustrate that microglial processes are highly motile in surveillance mode (left) and are instructed and directed by local neuronal activity to the most highly active neurons (middle, right). Microglia interact with adjacent neurons through neuronally released signaling molecules, monitoring and directing their activity. Microglial processes (red) engage with the soma of highly active neurons (orange), after which there is a decrease in both spontaneous and evoked neuronal firing. The findings illustrated are from live imaging in zebrafish larvae (see Li et al. [2012]).

(B) Microglia (red) drive programmed cell death during development and the apoptosis and removal of newborn neurons in the adult. Microglia not only phagocytose the debris of dead and damaged neurons but also instruct programmed cell death (PCD) via complement-mediated signaling events. A neuron destined for PCD is illustrated (in green) in the images; non-PCD-destined neurons are shown in gray. In the high-power cartoon images, a microglia process (red) is attracted to the green neuron (left). The neuron is subsequently engulfed and removed by the projecting phagocytic cup of local microglia (middle, right).

(C) Microglial activity drives the pruning, elimination, and maturation of synapses. The images on the upper-left illustrate low- and high-power views of microglia (iba1, red) and neuronal cell bodies (NeuN, green) in the mouse hippocampus. Below is a schematic showing the microglia (red) in close proximity to neuronal synaptic structures (green; enclosed in gray box). The potential influence of microglia on synaptic structures and two possible fates are shown in the cartoon in the enlarged boxed region on the right. The series of images on the right shows how superfluous exuberant synapses are pruned and eliminated by microglial activity. This process involves activation of the classical complement cascade, with accumulation of C1q at targeted synapses leading to neuronal C3-microglial CR3 signaling (see inset) and subsequent synaptic engulfment of both pre- and postsynaptic structures (green) by microglia (red). Conversely, the series on the left shows how other appropriate synapses can be strengthened by a CX3CR1-dependent mechanism (see inset, top) and subsequent maturation through postsynaptic NMDAR subunit changes and AMPAR insertion (see insets, bottom).

Driving Programmed Cell Death in CNS Development

Microglia are present in the CNS beginning from midembryonic stage and could therefore conceivably play a role in many aspects of the subsequent development of the CNS. However, in animals lacking microglia, such as PU.1 (Beers et al., 2006; Kierdorf et al., 2013; Smith et al., 2013) or CFSR1 null mutants (Erblich et al., 2011) or CNS-TGFβ-1 nulls (Butovsky et al., 2014), the gross architecture, regionalization, cell layering, and axonal tracks of the CNS develop normally. Whether microglia simply have no role in the major mapping of the CNS or whether they are not yet prepared or actively suppressed when this structuring develops is unknown, but it is clear that microglia are dispensable for the broad strokes of CNS development in the prenatal period. By contrast, during the postnatal period, microglia play critical roles in CNS development, refinement, and sculpting.

Because microglia are phagocytic, it has long been assumed that their primary role in CNS development is to engulf and clear the corpses of neurons that die as a result of programmed cell death, which eliminates the excess of neurons that are generated as part of normal development (Marín-Teva et al., 2011). However, microglia are not simply reactive waste collectors in development, but rather, they are active neuronal killers, assassinating neurons by inducing apoptosis. Several molecular mechanisms by which microglia can directly instruct neuronal apoptosis have been identified in different CNS regions (Figure 2B): (1) by releasing superoxide ions in the cerebellum (Marín-Teva et al., 2004), (2) through nerve growth factor in the retina (Frade and Barde, 1998), or (3) through tumor necrosis factor α (TNFα) in the spinal cord (Sedel et al., 2004). In the hippocampus of neonatal mice, microglia-induced neuronal apoptosis is dependent upon complement receptor 3 (CR3; comprised of CD11b/CD18, integrin α_M/integrin β_2) (Wakselman et al., 2008) and upon other cell surface receptors that transduce "kill-me" and "eat-me" signals (Schafer et al., 2013) expressed by neurons that are committed to die. Activation of these microglial cell surface receptors initiates intracellular downstream signaling through KARAP/DAP12, ultimately triggering the release of the

neurotoxic agents. The commitment of a particular neuron to die may be made cell autonomously. Alternatively, the microglial cells may also participate actively in the induction of kill-me and eat-me signals in the neurons (Marín-Teva et al., 2011). Numbers of neuronal precursors, as well as of differentiated neurons, are regulated by microglia (Cunningham et al., 2013). Thus, microglia regulate neuronal numbers in two important ways—controlling neuronal precursors as well as neurons.

Perhaps not surprisingly, the involvement of microglia in controlling neuron number is not as simple as being mediators of programmed cell death. Paradoxically, eliminating microglia is found to increase apoptosis of lamina V cortical neurons (Ueno et al., 2013). These neurons were found to require trophic support from microglia-derived IGF1. Whether this developmental role for microglia in maintaining rather than eliminating neurons is specific for lamina V cortical neurons or applies to neuronal subpopulations elsewhere in the CNS remains to be determined.

Synaptic Pruning in CNS Development

Not only does the developing CNS initially produce excessive numbers of neurons, the neuronal axons typically make exuberant synaptic connections to far more target neurons than are maintained in the fully developed nervous system. The projections are refined and sculpted to the mature architecture as large numbers of synapses are eliminated. Synaptic elimination—"synaptic pruning"—has long been known to be dependent on neuronal activity (Katz and Shatz, 1996), but over the past decade, microglia have emerged as critical for this process (Figure 2C). Indeed, in the cortex, the peak of microglial density coincides with the peak of synaptogenesis (Bessis et al., 2007). Developmental synaptic pruning has been extensively investigated in the retinogeniculate system, where the initial widespread and overlapping projections of retinal ganglion cells (RGCs) from both eyes are progressively segregated into stereotyped eye-specific territories (Schafer et al., 2013). Pruning of inappropriate RGC synapses in the lateral geniculate nucleus (LGN) is achieved by microglial engulfment of the synapses, both pre- and postsynaptic elements, in a retinotopically appropriate manner. The phagocytosis of the inappropriate synapses is lost in mice lacking complement receptor 3 (CR3), which is activated through the well-known classical complement cascade that begins with C1q and proceeds to opsonization of C3. In the CNS, CR3 is expressed only by microglia. Mice lacking C1q, CR3, or C3 have sustained defects in eye-specific segregation (Schafer et al., 2012; Stevens et al., 2007), implicating complement-dependent signaling as necessary for the synaptic elimination. Microglial engulfment of RGC-LGN inputs coincides with the period of postnatal development when C1q is expressed by RGCs. Using a retinal-specific deletion strategy (Bialas and Stevens, 2013) demonstrated that expression of C1q by RGCs depends upon their expression of TGFβ receptor II (TGFβRII). As eye-specific segregation and microglial engulfment of RCG-LGN synapses is lacking in mice with retinal-specific loss of TGFβRII, it appears that the requisite C1q comes from the RGCs themselves rather than from microglia, which express C1q continuously rather than phasically only during the period of synaptic pruning. C1q is transiently expressed as well in the developing neocortex and is necessary for proper elimination of exuberant synapses there (Chu et al., 2010). Thus, in the context of the complement system in the CNS, the signal to prune originates with the target neurons, but pruning is executed by the microglia.

Microglial engulfment of synaptic elements has also been observed in the developing hippocampus (Paolicelli et al., 2011). In mice lacking the chemokine receptor CX_3CR1, which in the CNS is only expressed by microglia, the number of microglia is transiently reduced in the developing hippocampus and synaptic pruning is delayed. This deficient synaptic pruning results in an excess of dendritic spines. Likewise, in the visual cortex, synapses are regularly contacted by microglia processes (Tremblay et al., 2010). A subset of synapses contacted by microglia disappears, suggesting that microglia may promote elimination of those in excess. Physiological synaptic pruning by microglia during development appears to be a common process for refining neuronal connectivity throughout the CNS. The phagocytosis of synaptic elements by microglia is a nonphlogistic process and, as such, is akin to phagocytosis by other tissue-resident macrophages such as the removal of aged erythrocytes by spleen red pulp macrophages (Davies et al., 2013) or engulfment of bacteria by macrophages in lamina propria of the gut (Danese, 2011). Therefore, developmental synaptic pruning by microglia has commonalities with noninflammatory processes of tissue-resident macrophages in homeostasis elsewhere in the body.

Microglia Are Critical in Synaptic Maturation

In addition to synaptic pruning, microglia are required for proper maturation of excitatory synaptic transmission. In CA1 hippocampal pyramidal neurons, the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs) are increased in CX_3CR1 null mice as compared with wild-type littermates around postnatal day 13 (P13) (Paolicelli et al., 2011). Mice lacking CX_3CR1 show persistent alterations in excitatory transmission, but no differences have been found with inhibitory synaptic transmission (Zhan et al., 2014). In addition, long-term depression (LTD) of excitatory synaptic transmission at developing Schaffer collateral-CA1 synapses is markedly enhanced in CX_3CR1 null mice. The impairments caused by lack of CX_3CR1 are suggestive of a role for microglia in maturation of synaptic functioning (Figure 2C). Consistent with a role of microglia in synaptic maturation, the functional properties of synapses develop abnormally in P18-25 mice carrying a mutation in KARAP/DAP12 (DAP12KI) (Roumier et al., 2004), a transmembrane receptor encoded by *TYROBP* and expressed by microglia. In the DAP12KI mice, glutamatergic synapses in the hippocampus have decreased expression of AMPA receptors, in particular the GluA2 subunit, compared with the wild-type. DAP12KI mice also show increased synaptic GluN2B NMDA receptor function, indicating loss of the normal developmental switch from GluN2B to GluN2A. Synaptic plasticity is also aberrant in DAP12KI mice with enhanced long-term potentiation (LTP), which may be attributed to a relative increase in calcium-permeable AMPA receptors due to the suppressed synaptic expression of GluA2.

In the whisker-barrel region of the somatosensory cortex, the "barrel" cortex, microglia are recruited to the regions of thalamocortical synapses, known as the barrel centers, around postnatal

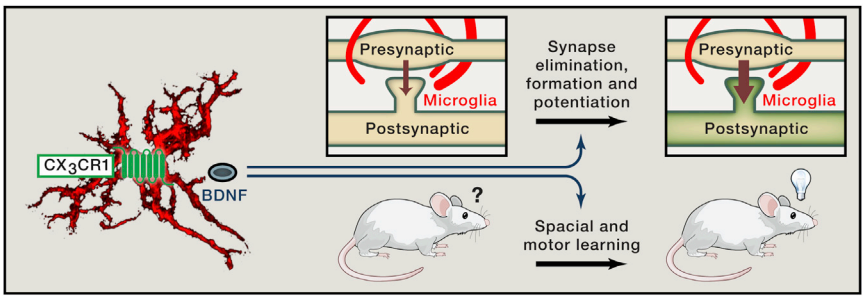

Figure 3. Microglial-Derived BDNF Drives Changes in Synaptic Formation Associated with Learning and Memory

Motor task learning (indicated by the cartoon on the right) is associated with changes in cortical synaptic dynamics, as indicated by synaptic maturation within the motor cortex (illustrated on the left side of the figure). Microglial-released BDNF (shown in blue being released from the microglia in red) influences the formation of synaptic structures in the motor cortex associated with the successful learning of a motor task. Genetic deletion of BDNF from CX_3CR1-expressing microglia leads to a reduction in dendritic spine dynamics and concomitant deficits in motor task learning (see Parkhurst et al. [2013]).

day 5. In CX_3CR1 null mice, the invasion of microglial cells to the barrel centers is delayed by several days, as is maturation of the thalamocortical synapses (Hoshiko et al., 2012). At these glutamatergic synapses, the ratio of the AMPA receptor-mediated:NMDA receptor-mediated component of the ESPC increases dramatically from P5 to P9. This increase is not observed in CX_3CR1 null mice. Over the same time period, there is a developmental switch in the composition of the synaptic NMDA receptor subunits, changing from predominantly GluN2B containing to predominantly GluN2A containing. This development switch appears absent or delayed in CX_3CR1 null mice. Thus, in both the hippocampus and barrel cortex and likely broadly in the CNS, proper microglia function is required for normal functional maturation of glutamatergic synapses.

Synaptic Plasticity and Learning in the Adult

What about synaptic functioning and plasticity in the normal adult CNS? In a number of mutant mouse strains in which microglia are lacking (e.g., CNS-TGFβ-1 null mice) or in which microglial signaling is disturbed (e.g., CX_3CR1 nulls), basal synaptic transmission and paired-pulse facilitation have been found to be indistinguishable from those in the wild-type (Koeglsperger et al., 2013; Rogers et al., 2011). Thus, although stimulating microglia can affect synaptic transmission, as administering LPS to stimulate microglia TLR4 receptors evokes increased frequency of mEPSCs and EPSCs (Pascual et al., 2012), unstimulated microglia in the healthy CNS have no demonstrable role in ongoing, basal synaptic function. Rather, it is during the processes of persistent synaptic plasticity when microglia come into play.

Persistent synaptic plasticity can be broadly conceptualized as either homeostatic—that which maintains synaptic function in the proper range—or activity triggered— that which alters synaptic efficacy, with the sign and magnitude of the alteration exquisitely dependent upon the frequency and patterning of synaptic inputs, postsynaptic neuronal firing, and synapse type. Homeostatic plasticity is known to require glial-derived TNFα (Stellwagen and Malenka, 2006). Though this cytokine may be released from microglia (Kettenmann et al., 2013), the specific role (if any) of microglia in homeostatic synaptic plasticity remains to be determined.

Activity-triggered, persistent changes in synaptic efficacy are commonly understood to be one of the fundamental cellular mechanisms underlying learning and memory. Such persistent, activity-triggered synaptic plasticity may manifest as either an increase or a decrease in synaptic efficacy, commonly referred to as long-term potentiation (LTP) or long-term depression (LTD). Learning and memory, particularly in the hippocampus, is also highly dependent upon the proliferation and development of new neurons—neurogenesis—which occurs in the healthy adult as well as in the developing CNS (Frankland et al., 2013; Shors et al., 2001). A recent spate of reports has put the spotlight on microglia as critical regulators of activity-triggered synaptic plasticity (Koeglsperger et al., 2013; Rogers et al., 2011; Schafer et al., 2013), adult neurogenesis (Bachstetter et al., 2011; Gemma and Bachstetter, 2013; Sierra et al., 2010), and learning/memory (Parkhurst et al., 2013; Rogers et al., 2011; Tremblay et al., 2011) (Figure 3). Microglia are also critical, through CR3, in a newly discovered form of LTD that is activity independent (Zhang et al., 2014).

The role of microglia in persistent, activity-triggered synaptic plasticity in the adult has been investigated in mice engineered to eliminate TGFβ from the CNS (Butovsky et al., 2014; Koeglsperger et al., 2013). In such CNS-TGFβ-1-deficient mice, the number of microglia is dramatically reduced beginning from E14.5, whereas neuron numbers are unaffected. The CNS-TGFβ-1-deficient animals are largely indistinguishable from controls until about P90, after which the mutants lose weight, exhibit dramatically reduced motor function, and die prematurely. Before observable changes have occurred, at 6–8 weeks postnatally, activity-triggered persistent synaptic plasticity is markedly altered in the hippocampus of the CNS-TGFβ-1-deficient mice. In these animals, LTP at Schaffer collateral-CA1 synapses is reduced, but conversely, LTD is enhanced. The profound alterations in synaptic plasticity are dependent upon enhanced activation of the GluN2B subtype of NMDA receptor and are attributed to reduced recycling of glutamate. Although it is possible that there are alterations in cells other than microglia, the hippocampal synaptic plasticity deficits in CNS-TGFβ-1 nulls are consistent with those from mice lacking CX_3CR1, in which LTP is virtually eliminated (Rogers et al., 2011). $CX_3CR1^{-/-}$ mice were also shown to have profound deficits in hippocampal-dependent learning and memory and in hippocampal neurogenesis.

The CNS-TGFβ-1-deficient mice lack CNS microglia from early in development, and CX_3CR1 is removed by a genomic deletion in the CX_3CR1 knockout mice. Thus, it is unclear as to whether the changes in synaptic plasticity are secondary to

developmental alterations or are due to alteration of synaptic plasticity per se in the adult. Another important issue for CX_3CR1 nulls and other mouse mutants of genes expressed commonly in the myeloid lineage (Figure 1) is that the primary manipulation will be not only in microglia, but also in circulating bone-marrow-derived macrophages. Both of these issues were addressed elegantly by Parkhurst et al. (2013), who generated mice expressing tamoxifen-inducible Cre recombinase under the control of the endogenous CX_3CR1 promoter, CX_3CR1^{CreER} mice. A similar tamoxifen-inducible Cre recombinase mouse was made by Yona et al., who focused on tissue-resident macrophages outside of the CNS (Yona et al., 2013) and microglia in CNS autoinflammation (Goldmann et al., 2013). To restrict Cre-mediated recombination to microglia, advantage was taken from the fact that microglia turn over at a slow rate, whereas CX_3CR1-expressing macrophages turn over rapidly and are replenished from bone marrow precursors that do not express CX_3CR1 (Fogg et al., 2006). In mice with CX_3CR1^{CreER}, Cre-mediated recombination persisted in microglia for more than a month after tamoxifen treatment, whereas the recovery in the macrophage population started within days of ending tamoxifen.

To probe necessary functions of microglia in the healthy adult brain, Parkhurst et al. crossed CX_3CR1^{CreER} mice with those carrying the Rosa26-stop-DTR ($R26^{iDTR}$) allele in which Cre-mediated excision of a stop codon derepresses expression of the diphtheria toxin receptor (DTR). Microglia, but not macrophages, were eliminated by treating CX_3CR1^{CreER}: $R26^{iDTR}$ mice first with tamoxifen and then 27 days later with diphtheria toxin. The microglia-depleted mice showed deficits in contextual fear conditioning and novel object recognition, tests of hippocampal-dependent learning, and in rotarod training, a test of motor learning. Motor learning is associated with increased spine dynamics in the motor cortex (Yang et al., 2009). By visualizing spine dynamics over days in vivo, it was found that microglia-depleted animals had reductions in both the elimination and the formation of spines induced by the motor learning paradigm. Importantly, the microglia elimination had no effect on brain levels of inflammatory cytokines, nor were there any signs of astrogliosis, problems that complicate the interpretation of other studies (Butovsky et al., 2014; Rogers et al., 2011). Thus, the findings by Parkhurst et al. are the strongest evidence to date for the essential role of microglia in synaptic plasticity and learning in the normal adult CNS.

In the coup de grace, an even more striking set of findings revealed that a single microglial-expressed protein may be responsible for most of the effects of microglia on synaptic plasticity and learning (Parkhurst et al., 2013). This protein is brain-derived neurotrophic factor (BDNF), a neurotrophin that is known to regulate synaptic plasticity (Chen et al., 1999; Lessmann, 1998; Nakajima et al., 2002; Stoop and Poo, 1996) and to be released by microglia (Trang et al., 2009). Treating CX_3CR1^{CreER} mice that were also homozygous for a floxed allele of BDNF with tamoxifen persistently and selectively eliminated BDNF from microglia. Mice in which BDNF was eliminated from microglia showed deficits in contextual fear conditioning and motor learning and in motor-learning-associated spine formation. Thus, microglial-derived BDNF is necessary for normal learning and spine plasticity. Loss of BDNF recapitulates many aspects of the synaptic plasticity and learning deficits caused by the overall depletion of microglia. No changes were found in microglia number, distribution, or proliferation or in the association of microglia processes with spines, which might have accounted for the deficits. Microglia-derived BDNF appears to act through its cognate receptor TrkB to regulate the level of synaptic GluN2B NMDA receptors. Eliminating BDNF expression by microglia had no measurable effect on the total BDNF content of the cortex or hippocampus, leading to the question as to why the massive expression of BDNF from nonmicroglial cells did not mask or compensate for the loss from microglia. BDNF is observed in puncta in processes of microglia (Trang et al., 2009), and as microglia processes may be preferentially drawn to active synapses, perhaps BDNF is released very locally precisely where it is needed.

With the recent series of findings from many labs using many approaches that demonstrate the uniqueness of microglia and the necessity of microglia and microglia-specific signaling processes in synaptic plasticity, learning, and neurogenesis, there seems to be no turning away from the conclusion that microglia are indispensable for the normal, physiological functioning of the adult CNS. Many intriguing new questions have arisen. If not BDNF, what accounts for the deficit in novel object recognition? Is learning-associated synapse elimination dependent on complement-mediated phagocytosis in the adult? Is microglial-derived BDNF important in adult neurogenesis? If not, what factor(s) is/are?

CNS Disorders when Noninflammatory Functions of Microglia Go Awry

Those questions and no doubt many others will drive research to expand our understanding of the breadth and depth of involvement of microglia in the healthy CNS. Nevertheless, even from the current understanding of microglia involvement, one may hypothesize that aberrations in noninflammatory functions of microglia, separate from their inflammatory functions, may contribute to disease processes in the CNS.

Complement-Dependent Phagocytosis and Pruning in Alzheimer's Disease

The growing knowledge of the fundamental role of microglia in synaptic elimination during development has important implications for understanding the mechanisms underlying synapse elimination and dysfunction in the diseased CNS. Synapse loss and dysfunction have emerged as early hallmarks of neurodegenerative diseases, suggesting that aberrant activation of complement upregulation may reactivate the developmental synapse elimination pathway to initiate synaptic loss. In particular, C1q associates with plaques in Alzheimer's brains from individuals with Alzheimer's disease (AD) (Afagh et al., 1996), and in mouse models of AD, C1q deficiency has been shown to be neuroprotective (Fonseca et al., 2004).

Recently, complement-dependent signaling has independently come to the forefront of AD research using a completely different type of approach to identify gene regulatory networks for late-onset Alzheimer's disease (LOAD) (Zhang et al., 2013). This approach yielded an immune/microglia module as the molecular system most strongly associated with the

pathophysiology of LOAD. Within this module, they identified a major gene subnetwork tightly linked to complement. At the center of this subnetwork is *TYROBP*, which encodes a transmembrane-signaling protein containing an immunoreceptor tyrosine-based activation motif (ITAM). *TYROBP* is directly coupled to *ITGAM*, the gene encoding CR3, and only two linkage steps from C1q. Also, microglia-expressed TYROBP (KARAP/DAP12) has been found to be directly involved in amyloid-β turnover and neuronal damage.

In other work, Griciuc et al. (2013) have found that human AD brain tissue contains excess CD33, an immunoglobulin-like cell surface protein, expressed in microglia. CD33 had been genetically linked to AD (Naj et al., 2011) and was linked to *TYROBP* in Zhang et al. (2013). Griciuc et al. (2013) found that CD33 inhibited uptake and clearance of Ab42 in microglial cell cultures and that loss of CD33 reduces amyloid plaque burden in a severe mouse model of AD, suggesting that dysregulation of CD33 plays a role in disease pathogenesis. Thus, the constellation of recent discoveries, including elucidation of the first gene network for late-onset AD, is shifting the focus to the pathophysiological roles of hijacking normal developmental processes in microglia.

Microglial Phagocytosis in Rett Syndrome

The critical role of microglial phagocytosis in the adult has been brought into focus by work on mouse models of Rett syndrome, an autism spectrum disorder caused by loss of the transcriptional factor MeCP2 (Amir et al., 1999). Whereas selective loss of MeCP2 in neurons recapitulates the full knockout phenotype, the return of MeCP2 function in microglia surprisingly rescued major phenotypic abnormalities caused by lack of MeCP2 (Derecki et al., 2012). MeCP2-deficient mice in which microglia MeCP2 function was reconstituted either by wild-type bone marrow transplant following irradiation or by microglia expression driven by Lysm-cre showed a dramatic improvement in lifespan, breathing patterns, and locomotor activity compared with mice transplanted with bone marrow from MeCP2-deficient mice. Phagocytosis was implicated as critical in the rescue because infusion of annexin V, which prevents recognition of debris by phagocytes, prevents the beneficial effects of restoring MeCP2.

Hoxb8+ Microglia: Link to a Compulsive Disorder

Hierarchical clustering analysis, which so dramatically demonstrates the differences between microglia and macrophages (Butovsky et al., 2014), has suggested that there may be microglial subpopulations with distinct transcriptomes. This concept builds on numerous studies showing functional, morphological subpopulations of microglia in different CNS regions (Scheffel et al., 2012; Vinnakota et al., 2013). One transcriptionally unique subpopulation of interest is that expressing the transcriptional factor Hoxb8. Hoxb8-expressing cells represent about 15% of the CD11b+ population in the adult CNS and may be ontogenically distinct from the yolk-sac-derived dominant population of microglia (S. De et al., 2013, Soc. Neurosci., abstract). Mice with complete loss of Hoxb8 or specific deletion in Tie2-expressing cells (myeloid and endothelial cells) show excessive grooming, leading to hair loss and skin lesions (Chen et al., 2010). The behavioral alterations are rescued by transplanting bone marrow from mice expressing Hoxb8 following irradiation, suggesting that reconstitution of Hoxb8+ myeloid is needed for normal grooming behavior. The excessive grooming behavior in mice is reminiscent of the human obsessive-compulsive disorder trichotillomania, raising the possibility that defects in the normal function of Hoxb8 in microglia may play a role in this disorder.

Microglia BDNF Signaling in Neuropathic Pain

Altered neuron-microglia-neuron signaling is increasingly recognized as being a core dysfunction in pain hypersensitivity after injury to peripheral nerves (Beggs et al., 2012; Tsuda et al., 2003, 2013). A major node in the core signaling network for nerve-injury-induced pain hypersensitivity is the release of BDNF from microglia in the spinal cord (Coull et al., 2005), the effects of which are mediated through TrkB receptors, leading to disinhibition of pain-transmitting neurons in the dorsal horn. Generalizing from the findings of Parkhurst et al. (2013), the pain hypersensitivity process may be an exaggeration of the physiological action of microglia-derived BDNF in learning and plasticity but in the context of a different neuronal network. In the motor cortex, microglia-derived BDNF is needed to facilitate the output of the motor programing network, resulting in enhanced motor performance. When microglia-derived BDNF facilitates the output of the dorsal horn pain-transmitting network, the result is enhanced pain.

Many aspects of the signaling pathways upstream and downstream from BDNF in microglia have been worked out in the context of nerve-injury-induced pain (Beggs et al., 2012). Downstream, after being released from microglia, BDNF activates neuronal TrkB receptors, leading to downregulation of the potassium-chloride cotransporter KCC2, with resultant accumulation of intracellular Cl^- in the neurons and suppressed inhibition (Coull et al., 2003). Whether lessening of Cl^--mediated inhibition participates in the physiological plasticity and learning that depend upon microglia-derived BDNF in the adult is unknown. During development, intracellular Cl^- is elevated, and KCC2 expression is low, contributing to heightened synaptic plasticity (Rivera et al., 1999); thus, it is possible that microglia-derived BDNF could play a role here. The dominant upstream pathway for microglial BDNF in the context of pain hypersensitivity is activation of P2X4 receptors, which causes increased intracellular Ca^{2+} and activation of p38 MAP kinase. This signaling pathway drives transcription, translation, and release per se of BDNF from microglia (Trang et al., 2009). Expression of P2X4 receptors by the microglia is, in turn, driven by convergence of a number of signaling cascades involving integrin and chemokine receptors that are initiated following nerve injury (Biber et al., 2011; Masuda et al., 2012; Toyomitsu et al., 2012; Tsuda et al., 2009a, 2009b). Importantly, the site of injury in the peripheral nerve is far removed from the microglia response in the spinal cord, and hence this is not an inflammatory response to local injury.

The P2X4 receptor-BDNF-TrkB-KCC2 pathway expression is not only critical for pain hypersensitivity after peripheral nerve injury, but also for the paradoxical hyperalgesic effect of morphine and other opioids (Ferrini et al., 2013). Thus, exaggeration of the physiological plasticity induced by microglia-derived BDNF may have a number of pathological sequelae. Conversely, suppression of BDNF from microglia may contribute to disorders in which there is reduced plasticity, such as schizophrenia (Buckley et al., 2011) or AD (Nisticò et al., 2012).

Concluding Remarks

The recent flood of evidence demonstrates that microglia are a specialized cell population that have multiple roles in shaping and refining neuronal connectivity during development and in activity-dependent synaptic plasticity, neurogenesis, and learning in the fully mature CNS. Microglia can detect, process, and respond to signals in an entirely noninflammatory way. The duality of microglia with noninflammatory as well as inflammatory functions provides a challenge to the concept that all disorders involving microglia are de facto neuroinflammatory disorders. Rather, some CNS disorders, or endophenotypes of disorders, may better be conceptualized as resulting from loss or gain of noninflammatory microglia functions. We anticipate that advancing understanding of these functions of microglia will not only deepen knowledge of fundamental CNS mechanisms, but will also reveal new strategies for diagnosing and treating CNS disorders.

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