MICROGLIA: expanding roles for the guardian of the CNS

Our view of microglia has dramatically changed in the last decade. From cells being “silent” in the healthy brain, microglia have emerged to be actively involved in several brain physiological functions including adult hippocampal neurogenesis, and cognitive and behavioral function.

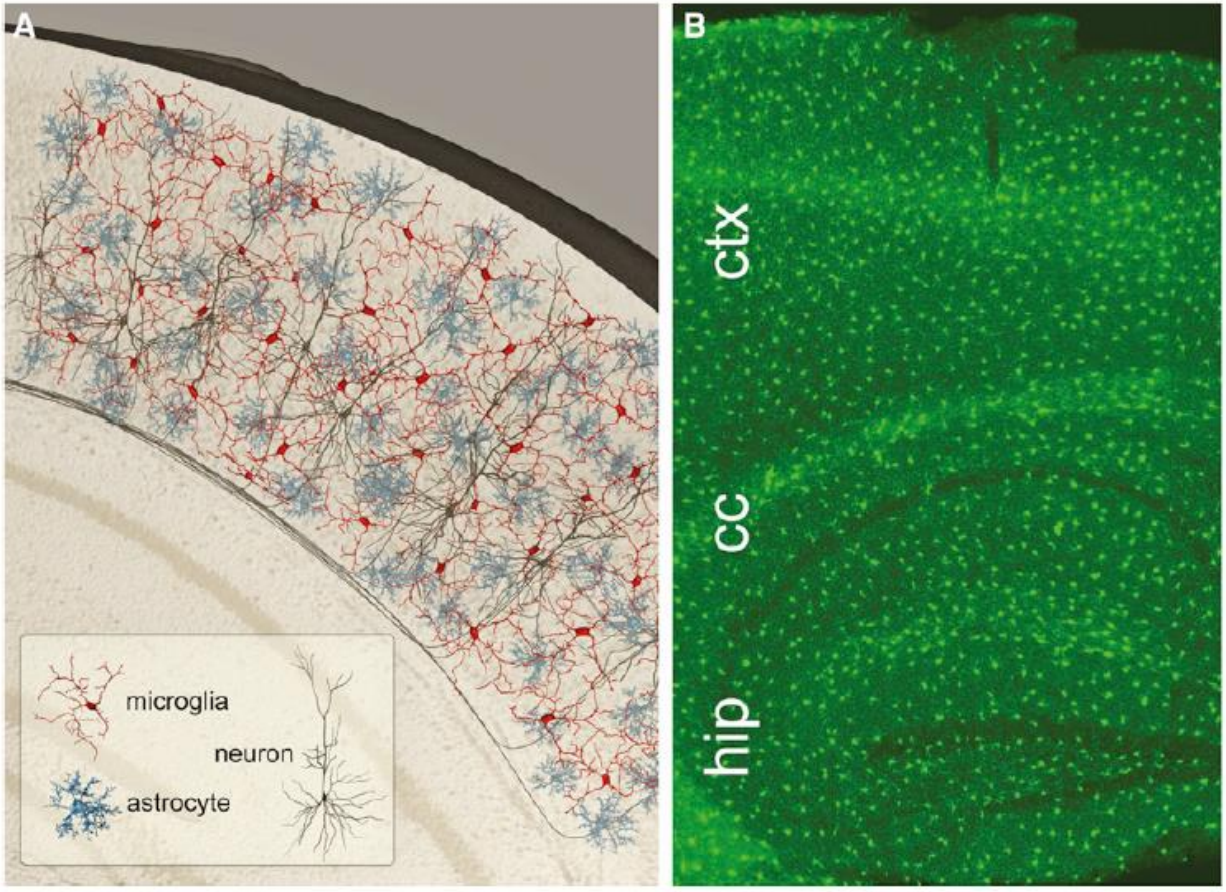

Figure 1. Uniform Distribution of Microglia in the Central Nervous System

(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).

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Microglia originates from a pool of primitive macrophages from the yolk sac that appear in the mouse at embryonic (E) day 8.5 and invade the brain from E9.5. These cells constitute an independent lineage distinct from other haematopoetic stem cells.

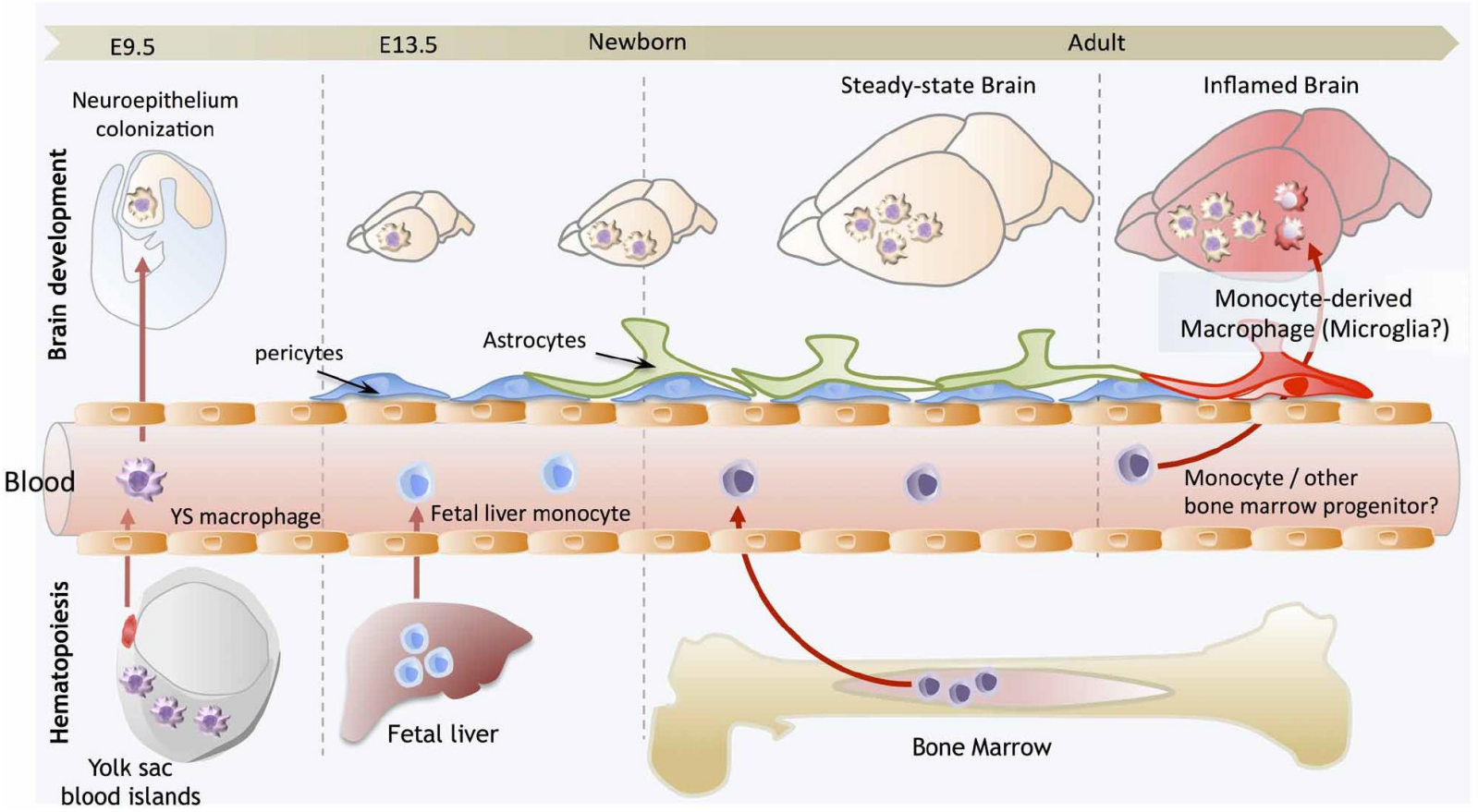
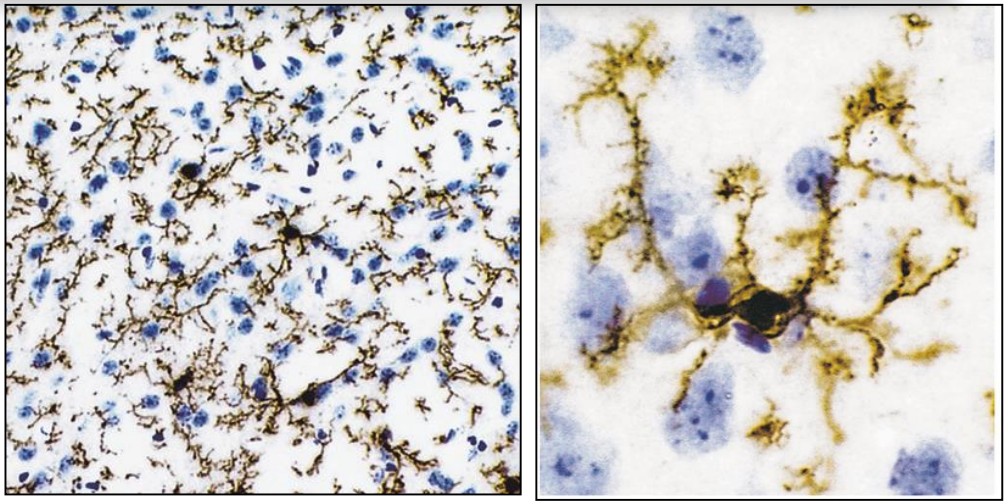


FIGURE 1 | Brain development and microglial homeostasis. Primitive macrophages exit the yolk sac blood islands at the onset of circulation and colonize the neuroepithelium from E9.5 to give rise to microglia. The blood brain barrier starts to form from E13.5 and may isolate the developing brain from the contribution of fetal liver hematopoiesis. Embryonic microglia expand and colonize the whole CNS until adulthood. Importantly, in steady state conditions, embryonically-derived microglia will maintain themselves until adulthood, via local proliferation during late gestation and post-natal development as well as in the injured adult brain in reaction to inflammation. Nevertheless, during certain inflammatory conditions found for example after bone marrow transplantation, the recruitment of monocytes or other bone marrow-derived progenitors can supplement the microglial population to some extent. However, we do not understand yet whether these cells persist and become integrated in the microglial network, or are a temporary addition to the endogenous population.

Ginhouks et al., 2013
doi: 10.3389/fncel.2013.00045

Microglia constantly move their processes to scan the brain parenchyma



«ramified microglia»

Figure 4–19 Large numbers of microglia reside in the mammalian central nervous system. The micrograph on the left shows microglia in the cerebral cortex of an adult mouse (in **brown**, immunocytochemistry). The **blue** spots are the nuclei of nonmicroglial cells. The microglial cells have fine, lacy processes, as shown in the higher magnification micrograph on the right. (Reproduced, with permission, from Berry et al. 2002.)

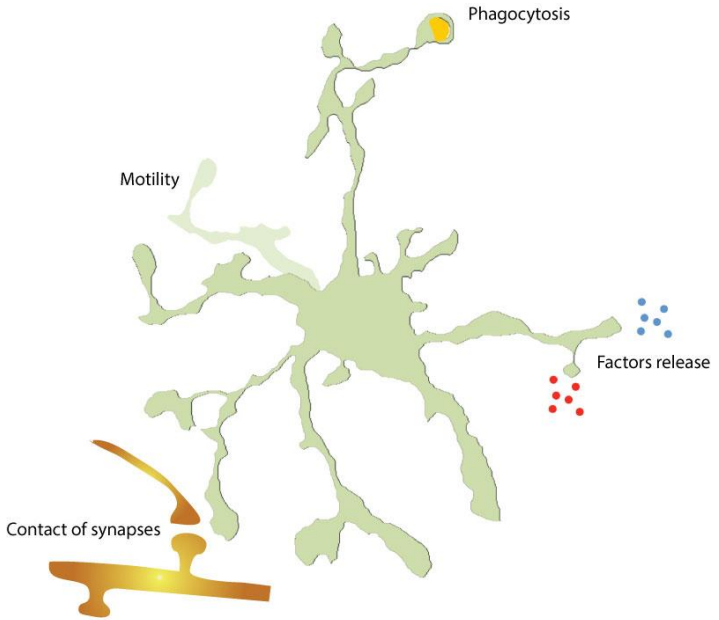
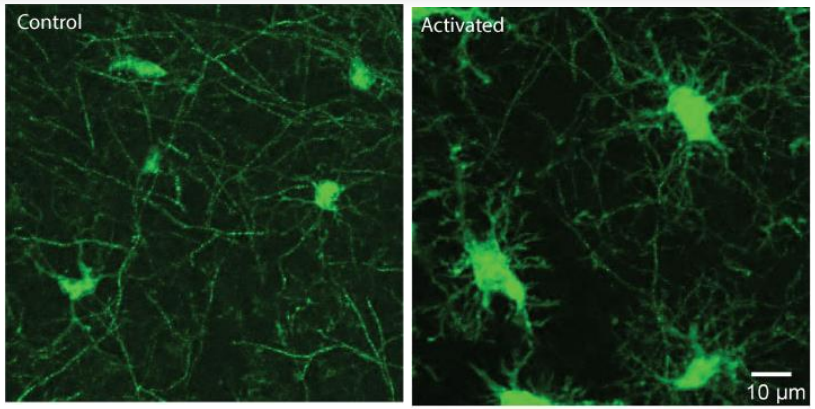


Figure 2.6 Scheme of the different functions of microglia.
Microglia (green) constantly move their processes to scan the brain parenchyma. During their movements they contact synapses and neuronal dendrites (orange), as well other brain cells. They can control brain activity and surrounding cells' fate by releasing several factors. They phagocytose cells and neuronal debris, but also synaptic elements and newborn cells (orange), thus they participate in sculpting the neuronal circuits.
Drawing by E. Avignone.

Morphological change following microglial activation



«ramified microglia» «reactive microglia»

Figure 2.7 Microglia change properties after activation. The images show an example of morphological changes of microglia 48 hours after activation induced by *status epilepticus*. In control conditions (**a**) microglial cells have a small body with long and ramified processes. (**b**) In contrast, activated microglial cells have larger body with shorter and thicker processes.
From Menteyne A, Levavasseur F, Audinat E, Avignone E (2009) Predominant functional expression of Kv1.3 by activated microglia of the hippocampus after status epilepticus. *PLoS One* 4, e6770, with permission.

Microglial cells respond rapidly to injury by migrating to the damaged site

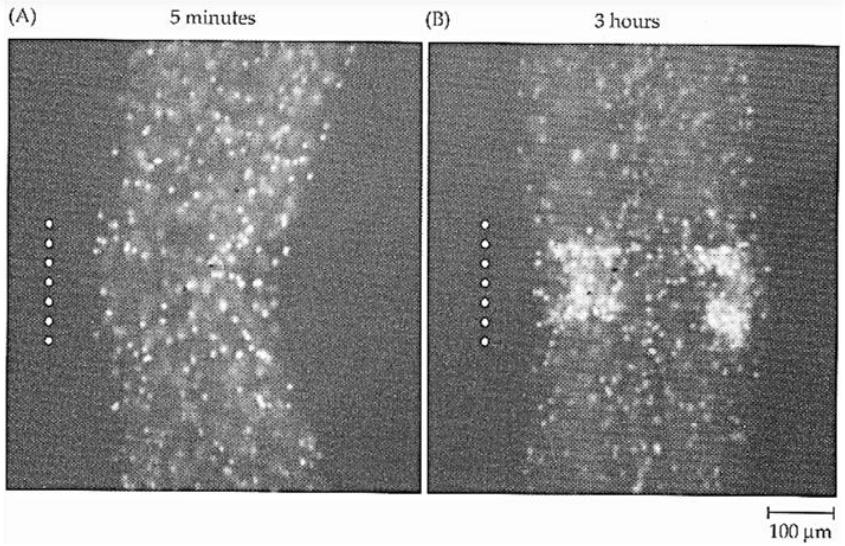
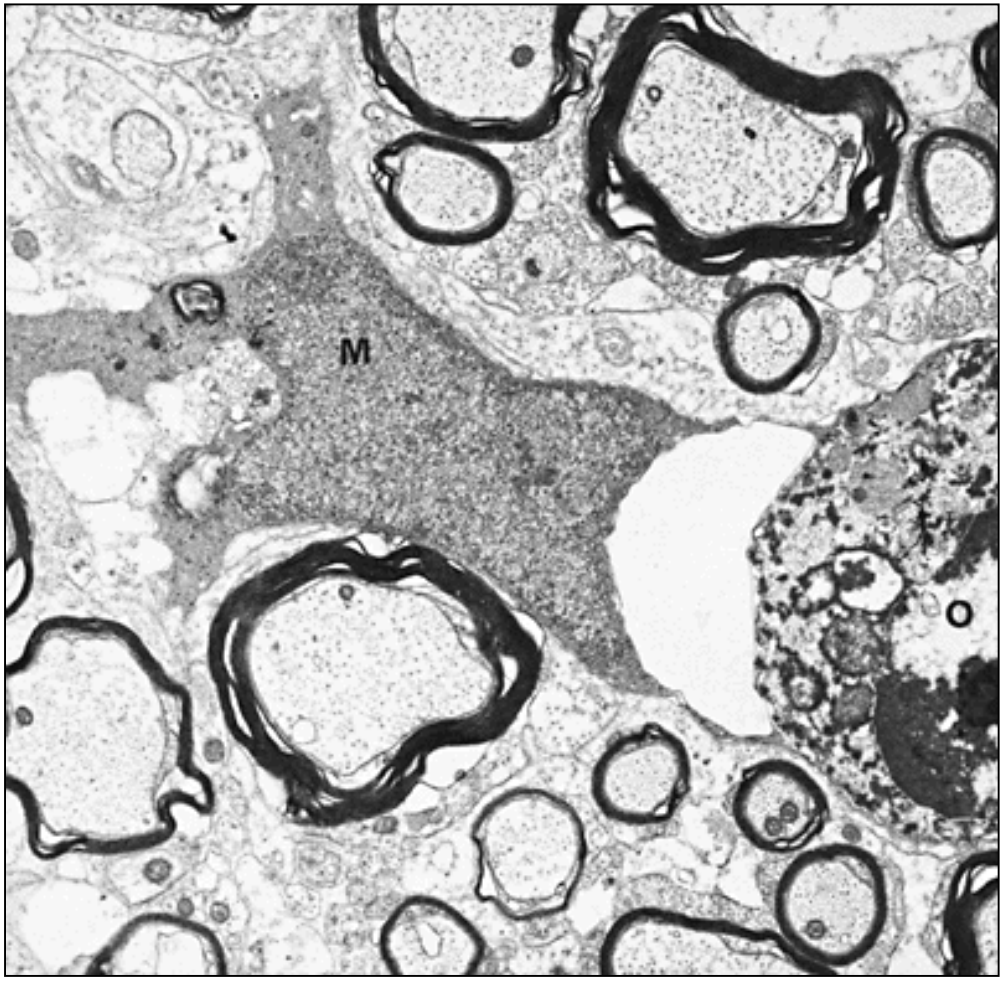


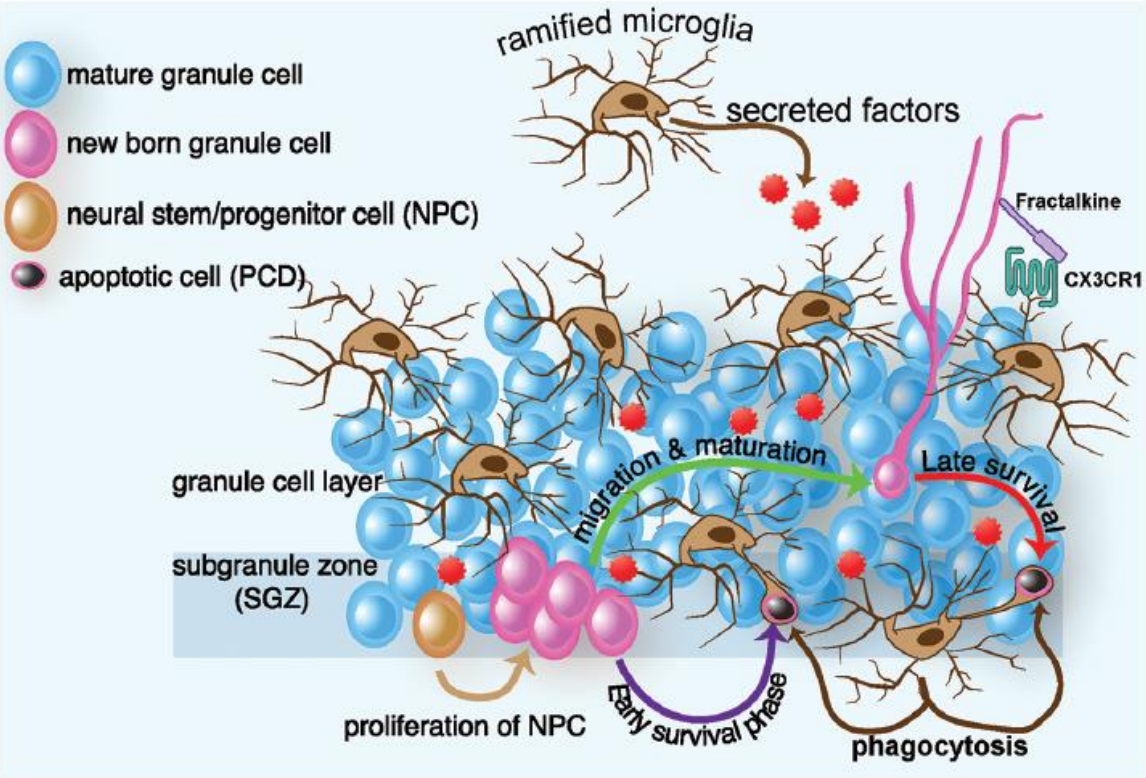
FIGURE 8.11 Migration of Microglial Cells in Injured CNS. (A) Microglia in the leech CNS were stained with a fluorescent nuclear dye (Hoechst 33342). The bundle of axons linking ganglia had been crushed 5 minutes earlier. The extent of the crush is indicated by the dotted line. The nuclei of microglial cells were still evenly distributed at this time. (B)Three hours after the injury, microglial cells had accumulated at the crush site. There they produced the growth-promoting molecule laminin. (C) Veloci-

Phagocytotic activity of microglia

A **microglial cell** (M) has elaborated two cytoplasmic arms to encompass a degenerating apoptotic oligodendrocyte (O) in the spinal cord of a 3-day-old kitten. The microglial cell nucleus is difficult to distinguish from the narrow rim of densely stained cytoplasm, which also contains some membranous debris. 10,000.



Microglia and hippocampal neurogenesis



Ramified microglia remove apoptotic neurons forming **phagocytic pouches** that engulf the apoptotic cells. The phagocytic pouches occurs independent from the cell body, in terminal or en passant branches, as opposed to engulfment of the soma by ameboid microglia.

FIGURE 1 | Schematic diagram of ramified microglia and their effect on adult hippocampal neurogenesis. In intact brain, microglia regulate several steps of adult hippocampal neurogenesis. In the SGZ, progenitor cells migrate to the granule cell layer and differentiate into a neuronal phenotype, with most NPCs dying in the first few days of life. Within two months, the surviving neurons receive input, form functional synapses with their target cells, and exhibit electrophysiological properties indistinguishable from those of mature neurons. In intact brain, ramified microglia eliminate apoptotic newborn cells during the first few days of their life by phagocytosis. This phagocytosis occurs by a special modification of the microglial processes, which form phagocytic pouches that engulf the apoptotic cells. Microglia can also affect proliferation, differentiation, and survival, through the secretion of neurotrophic factors. Finally microglia communicate with nearby neurons through the CX3CR1/CX3CL1 signaling. Interactions between CX3CL1 and CX3CR1 contribute to the ability of microglia to maintain a surveillant/ramified phenotype. Disruption of this signaling results in a change in microglia phenotype and function, which leads to decreased hippocampal neurogenesis.

Gemma & Banchstetter, 2013, Frontiers in Cellular Neuroscience
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Microglial cells can sense neuronal activity

It has recently become evident that they constantly scan the brain environment and contact synapses.

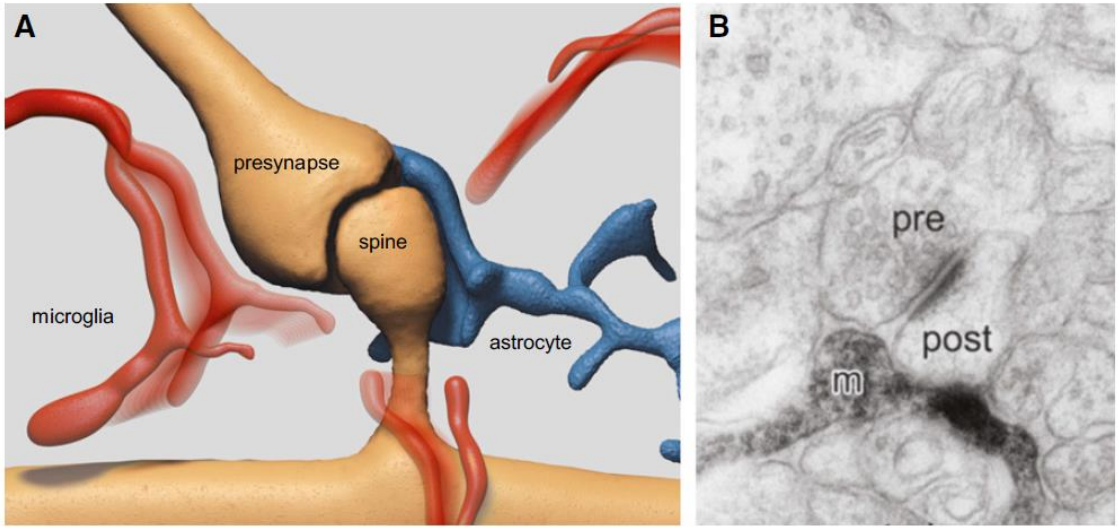

Figure 2. Dynamic Interaction of Microglial Processes with the Tripartite Synapse

(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).

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Activated microglia can remove damaged cells as well as dysfunctional synapses, a process termed "**synaptic stripping**"

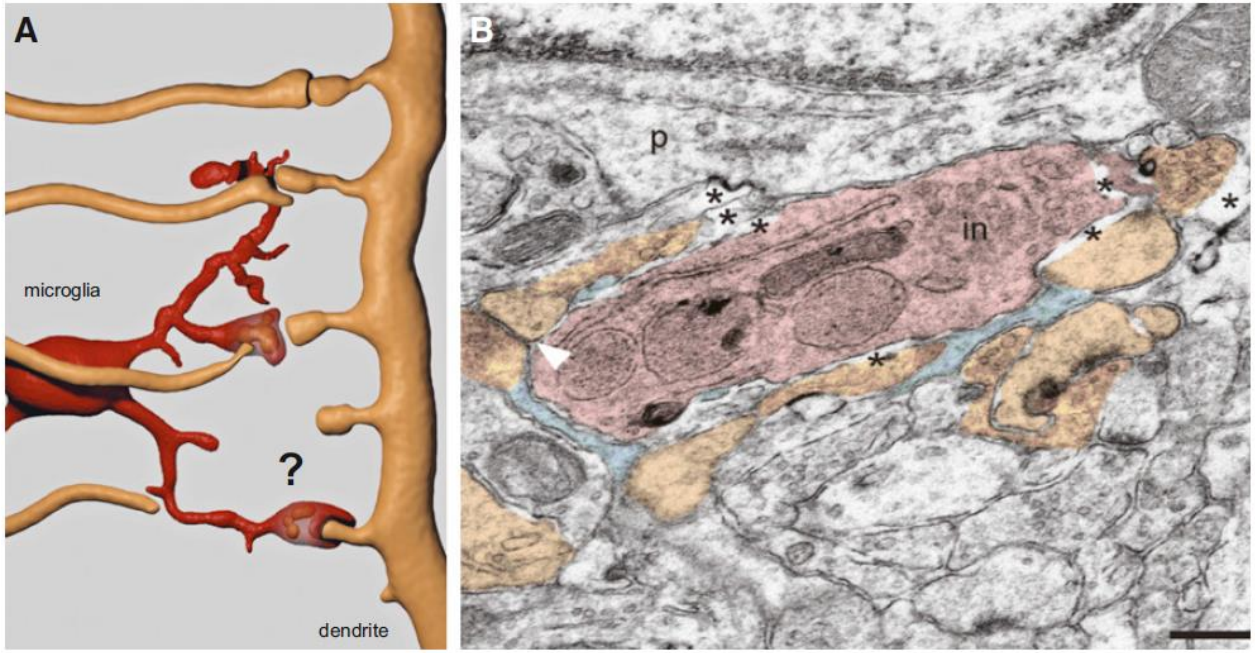

Figure 3. Synaptic Pruning by Microglial Processes

(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.

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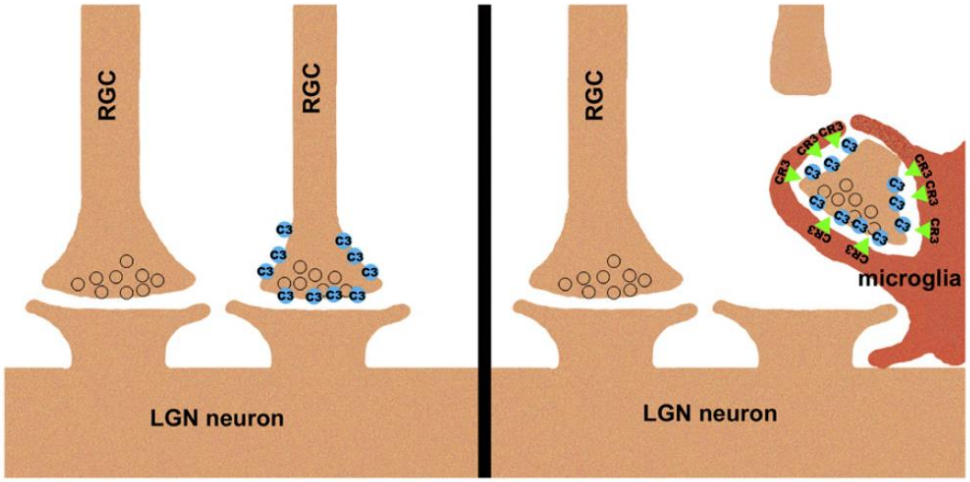
Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner

Shafer et al., 2012 Neuron 74, 691–705



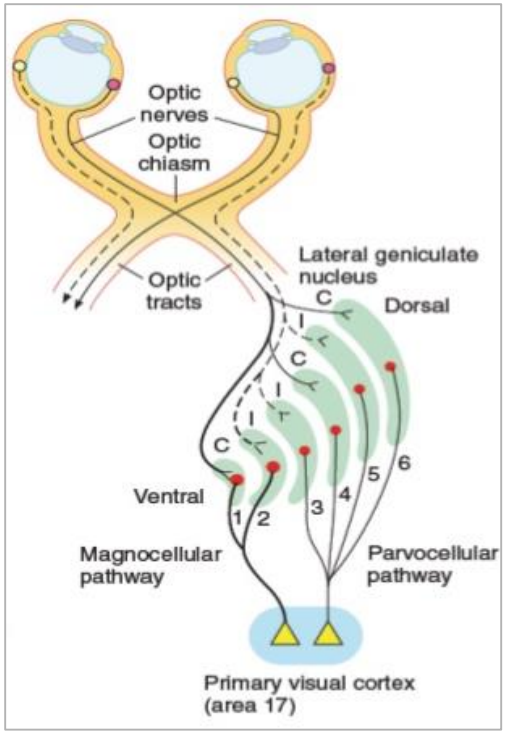


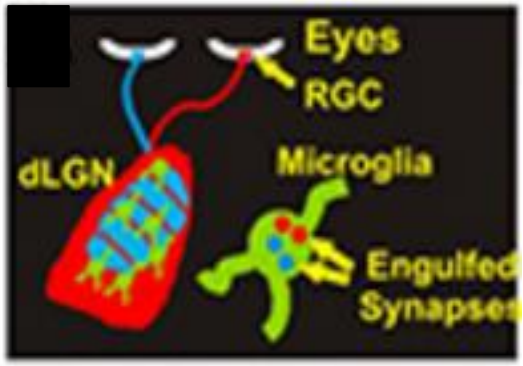
Figure 1. Microglia Phagocytose RGC Axon Material in a C3- and CR3-Dependent Manner

Proteins of the major histocompatibility complex class I (MHCI) and complement cascade (C1q and C3) are expressed in the developing brain and are necessary for normal pruning of **Retinal Ganglion Cells** (**RGC**) axons in the dorsal **Lateral Geniculate Nucleus** (**dLGN**). Schafer et al. demonstrate a role for microglia in activity-dependent synaptic pruning in the postnatal retinogeniculate system. They show that microglia engulf presynaptic inputs during peak retinogeniculate pruning and that engulfment is dependent upon neural activity and the **microglia-specific phagocytic signaling pathway**, **complement receptor 3(CR3)/C3**. The interpretation is that C3 serves as a **tag for synapses that need to be eliminated**.

VIDEO EXPERIMENT
An Engulfment Assay: A Protocol to Assess Interactions Between CNS Phagocytes and Neurons
Dorothy P. Schafer[1], Emily K. Lehrman[1], Christopher T. Heller[1], Beth Stevens[1]
***J. Vis. Exp.* (88), e51482, doi:10.3791/51482 (2014)**
http://www.jove.com/video/51482/an-engulfment-assay-protocol-to-assess-interactions-between-cns

A strategy to visualize left and right RGC nerve terminals in the LGN and their engulfment into microglial cells

RGCs from CX3CR1-EGFP eterozygous mice* were anterogradely traced with CTB-594 and CTB-647 into the left and right eyes, respectively. Following this tracing, EGFP-positive microglia within the dLGN were imaged.



* Microglia were labeled using the CX3CR1+/GFP mouse line in which all microglia express EGFP under the control of fractalkine receptor, CX3CR1

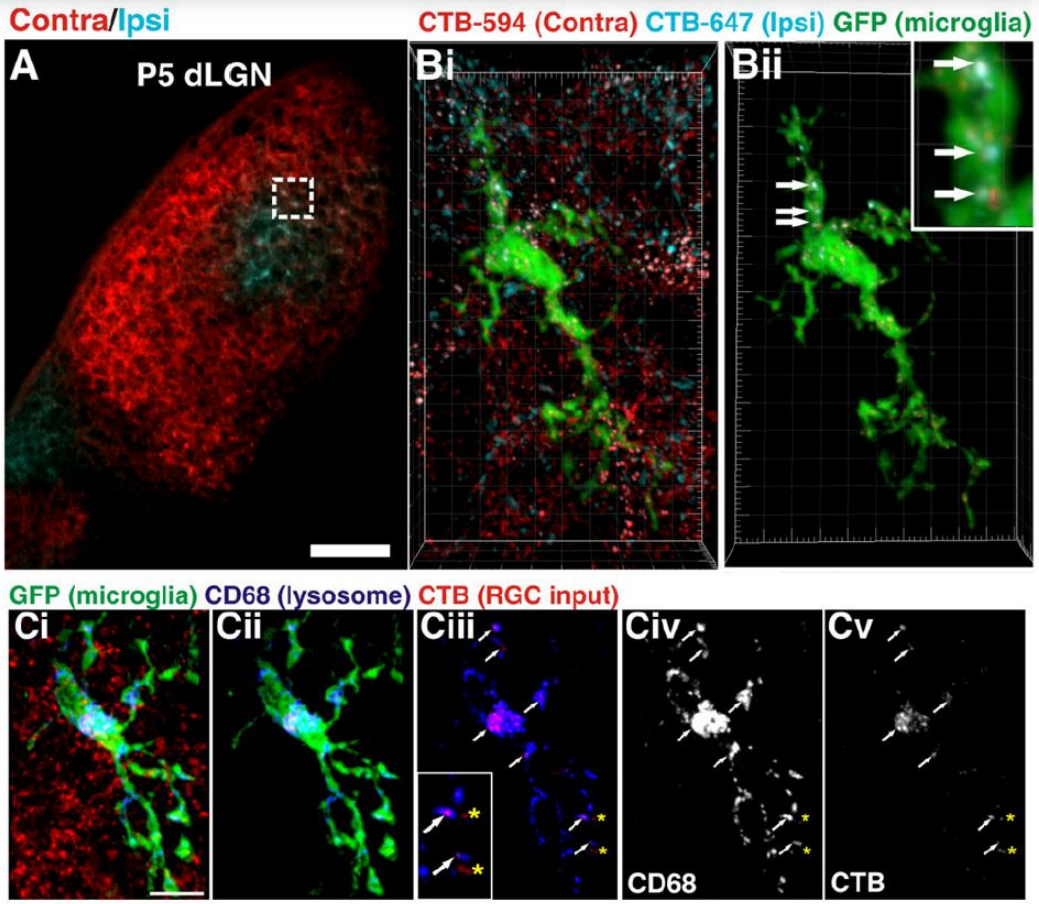
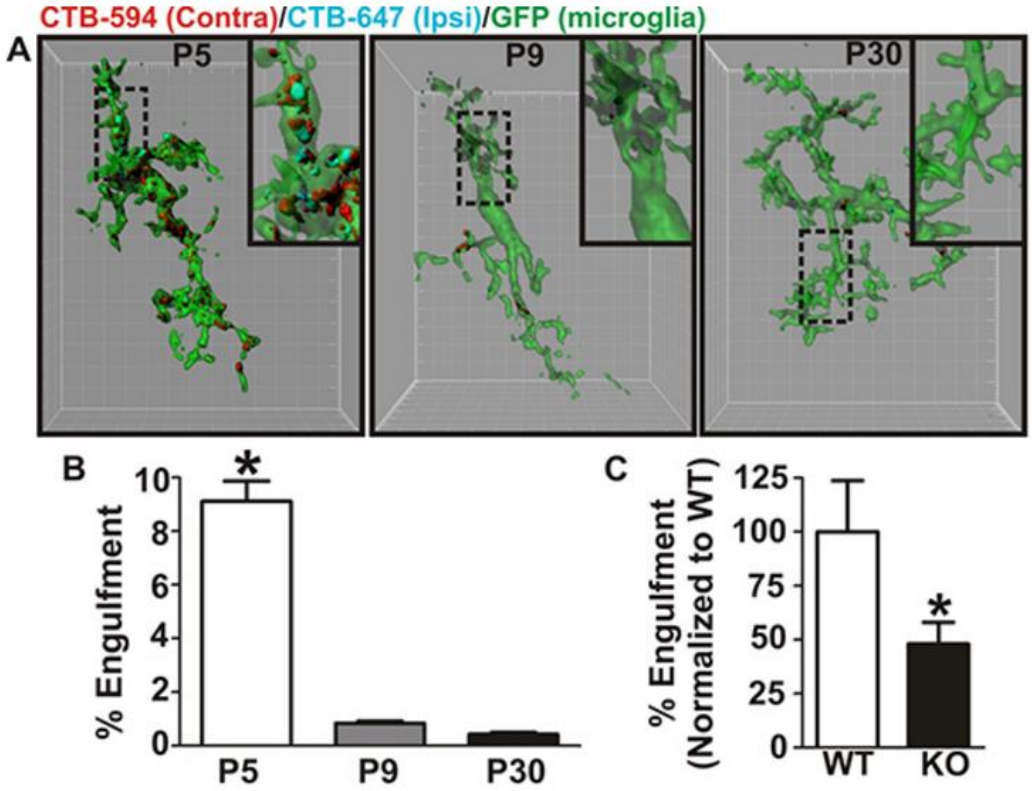


Figure 1. Microglia Engulf RGC Inputs Undergoing Active Synaptic Pruning in the dLGN

(A) A representative low-magnification image of P5 dLGN. Ipsilateral inputs are labeled with CTB-647 (blue) and contralateral inputs are labeled with CTB-594 (red). Scale bar = 100 μm.

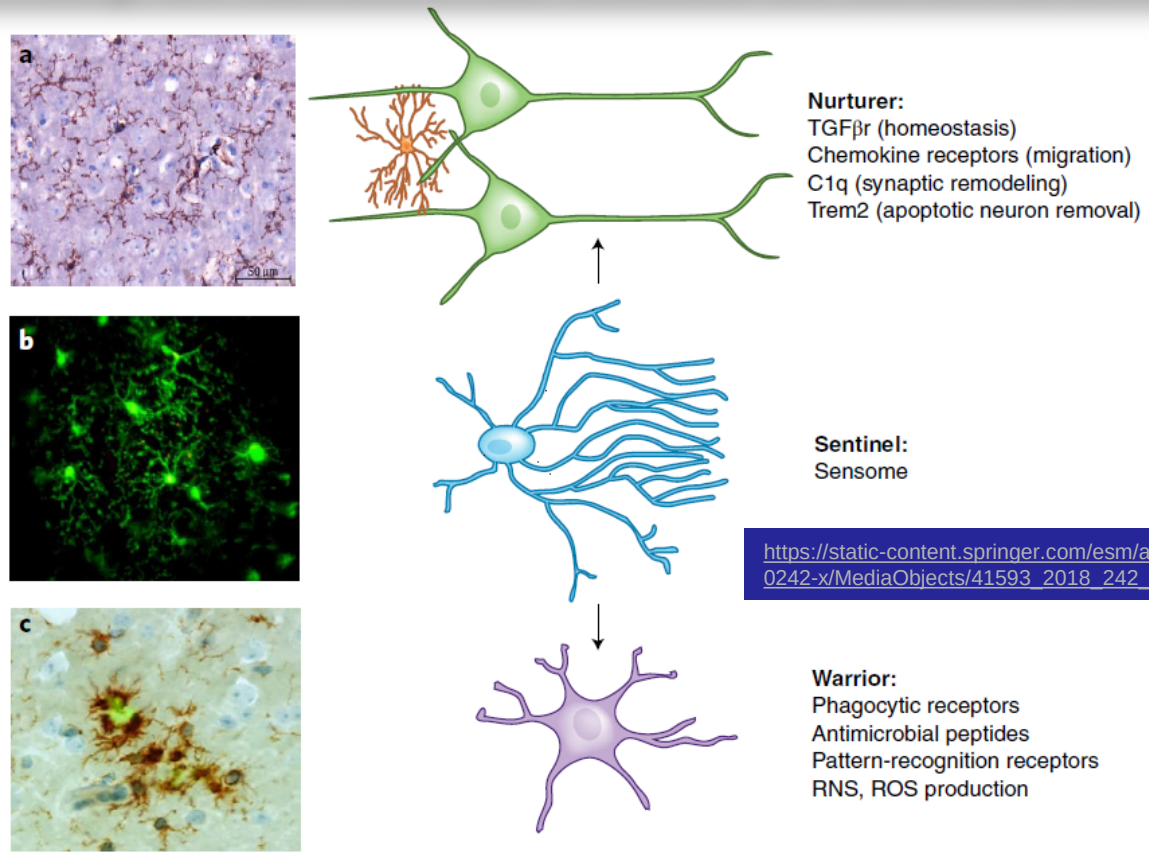
(Bi) A microglia (EGFP, green) sampled from the border region of ipsilateral (blue) and contralateral (red) projections (inset in A). (Bii) All CTB fluorescence outside the microglial volume has been subtracted revealing RGC inputs (red and blue) that have been engulfed (arrows, enlarged in inset). Grid line increments = 5 μm. (Ci) A representative microglia (green, EGFP) from P5 dLGN. RGC inputs from both eyes are labeled with CTB-594 (red) and lysosomes are labeled with anti-CD68 (blue). (Cii) The same microglia in which all CTB fluorescence outside the microglia volume has been removed revealing lysosomes (blue) and engulfed RGC inputs (red). (Ciii) The same cell in which only the lysosomes (blue) and RGC inputs (red) are visualized in which most inputs (red) are localized within CD68-positive lysosomes (blue; white arrows). There are few instances in which CTB is not localized to lysosomes (yellow asterisks). Inset is enlarged region of (Ciii). (Civ and Cv) The CD68 (Civ) and CTB (Cv) channels alone. Scale bar = 10 μm.



Images were subsequently surface-rendered for volume measurements.

A) Representative surface-rendered microglia from P5 (fluorescent image is shown in **Figure 1**), P9, and P30 mouse dLGN. Enlarged insets denoted with a black dotted line. Grid line increments = 5 µm. **B)** Engulfment of RGC inputs is significantly increased during peak pruning in the dLGN (P5) versus older ages (P9 and P30). *$P < 0.001$ by one-way ANOVA, n = 3 mice/age. **C)** Microglia from **mice deficient in complement receptor 3 (KO**, black bar) engulf significantly fewer RGC inputs as compared to WT littermates (white bar). All data are normalized to WT control values. *$P < 0.04$ by Student's *t*-test, n = 3 mice/genotype. All error bars represent s.e.m.

Summary of functional states of MICROGLIA



https://static-content.springer.com/esm/art%3A10.1038%2Fs41593-018-0242-x/MediaObjects/41593_2018_242_MOESM2_ESM.mp4

Fig. 2 | Three proposed functional states of microglia. a, Nurturer state: microglia (left) stained for Cd11b (brown) in a normal brain are highly ramified and evenly spaced throughout the brain parenchyma. In their nurturer role they maintain milieu homeostasis, participate in synaptic remodeling and migration, and remove apoptotic neurons, all mediated by specific receptors and receptor-linked pathways. **b**, Sentinel state: micrograph taken from a video using two-photon microscopy from a Cx3cr1-GFP mouse with a cranial window shows a cluster of green microglia with abundant processes. The video from which this micrograph was taken (Supplementary Video 1) shows that microglia (green) processes are in constant motion, surveilling their surroundings. Focal laser-induced injury initiates microglia response, with those microglia closest to the site of injury displaying polarization of surveilling processes toward the area of injury. Microglia sensing is mediated by proteins encoded by sensome genes, which are portals for microglia to perform their housekeeping and host-defense functions. **c**, Warrior state: microglia (left) stained for Cd11b (brown) accumulate around Aβ deposits stained with thioflavin-S (green), where they are observed to be two- to fivefold denser than in neighboring areas. The warrior morphology becomes stockier and less ramified, and defense against infectious pathogens and injurious-self proteins including Aβ is mediated through microglial Fc receptors, TLRs, viral receptors, and antimicrobial peptides. Sensing is a prerequisite for microglia to perform their housekeeping and host-defense functions.