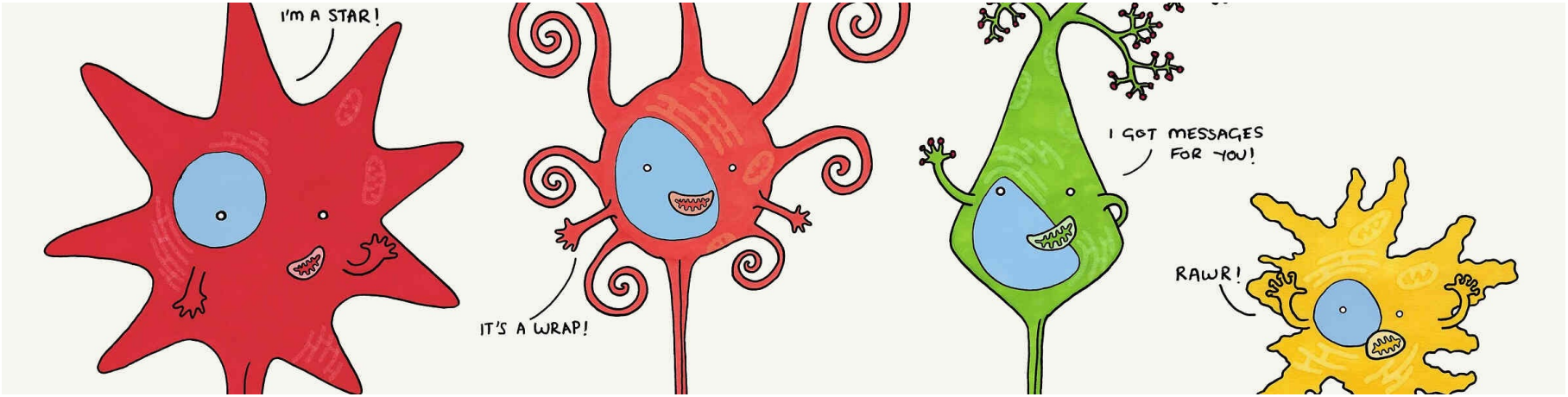
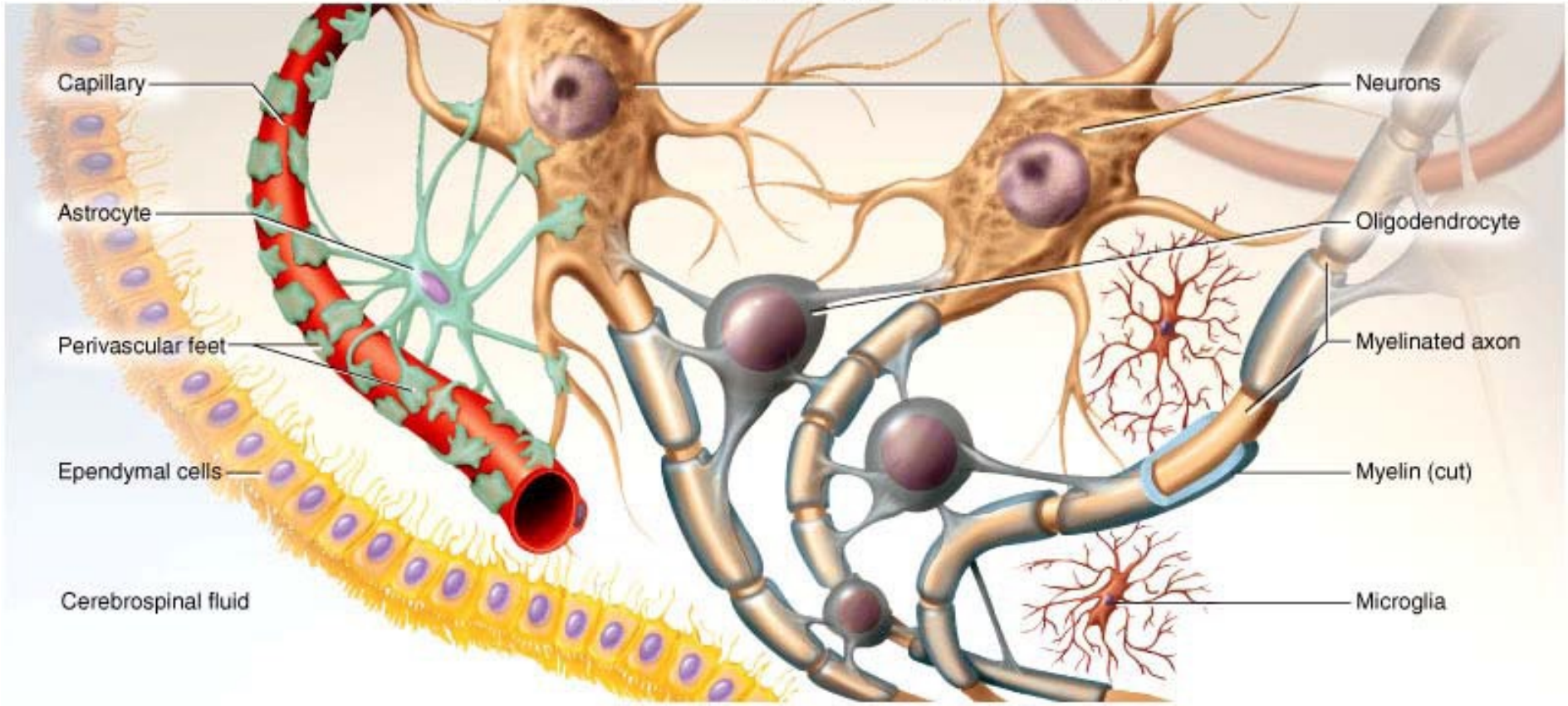
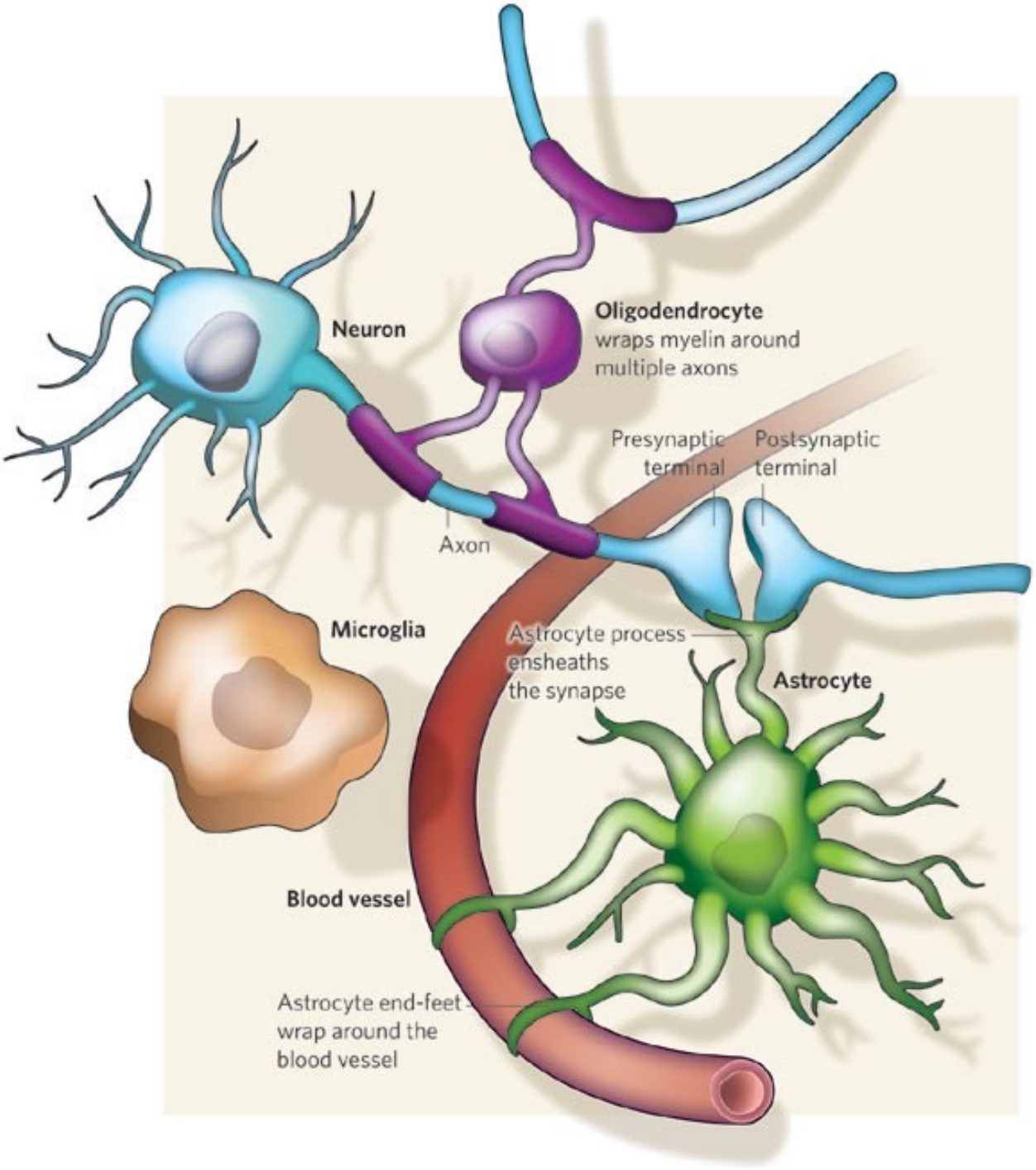


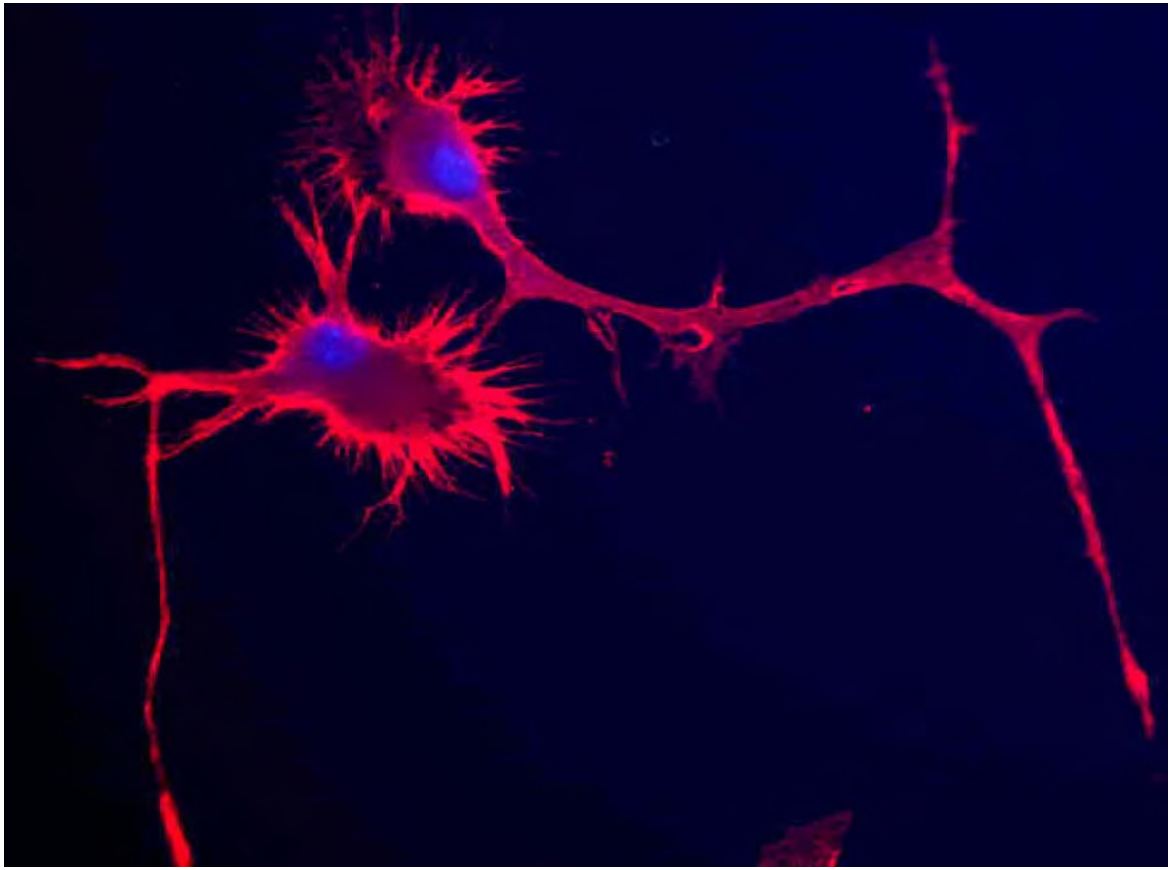
GLIA - Microglia

Neurocentric vision…..

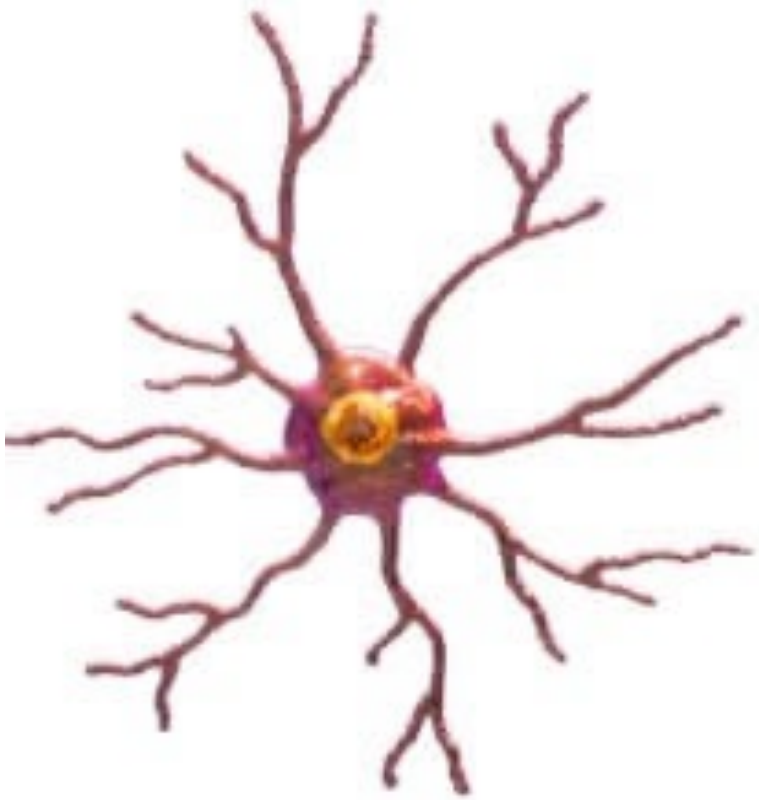


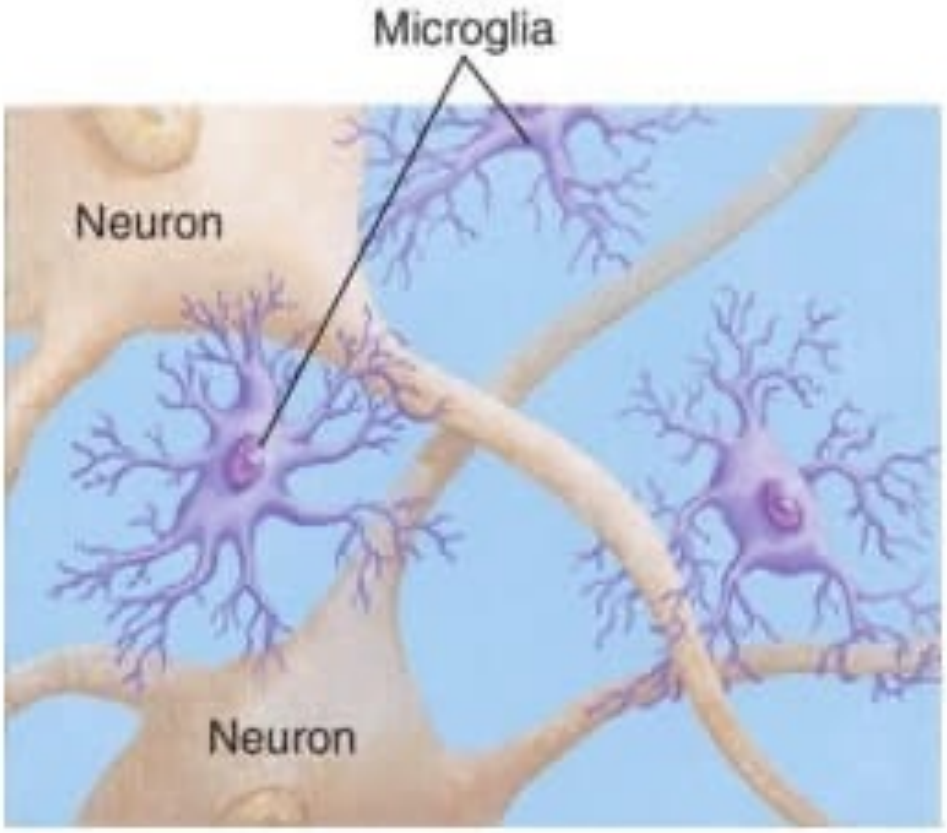

Neuron
Oligodendrocyte
wraps myelin around
multiple axons
Presynaptic
terminal
Postsynaptic
terminal
Axon
Microglia
Astrocyte process
ensheaths
the synapse
Astrocyte
Blood vessel
Astrocyte end-feet
wrap around the
blood vessel

Types of glia: microglia



Types of glia: microglia





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.

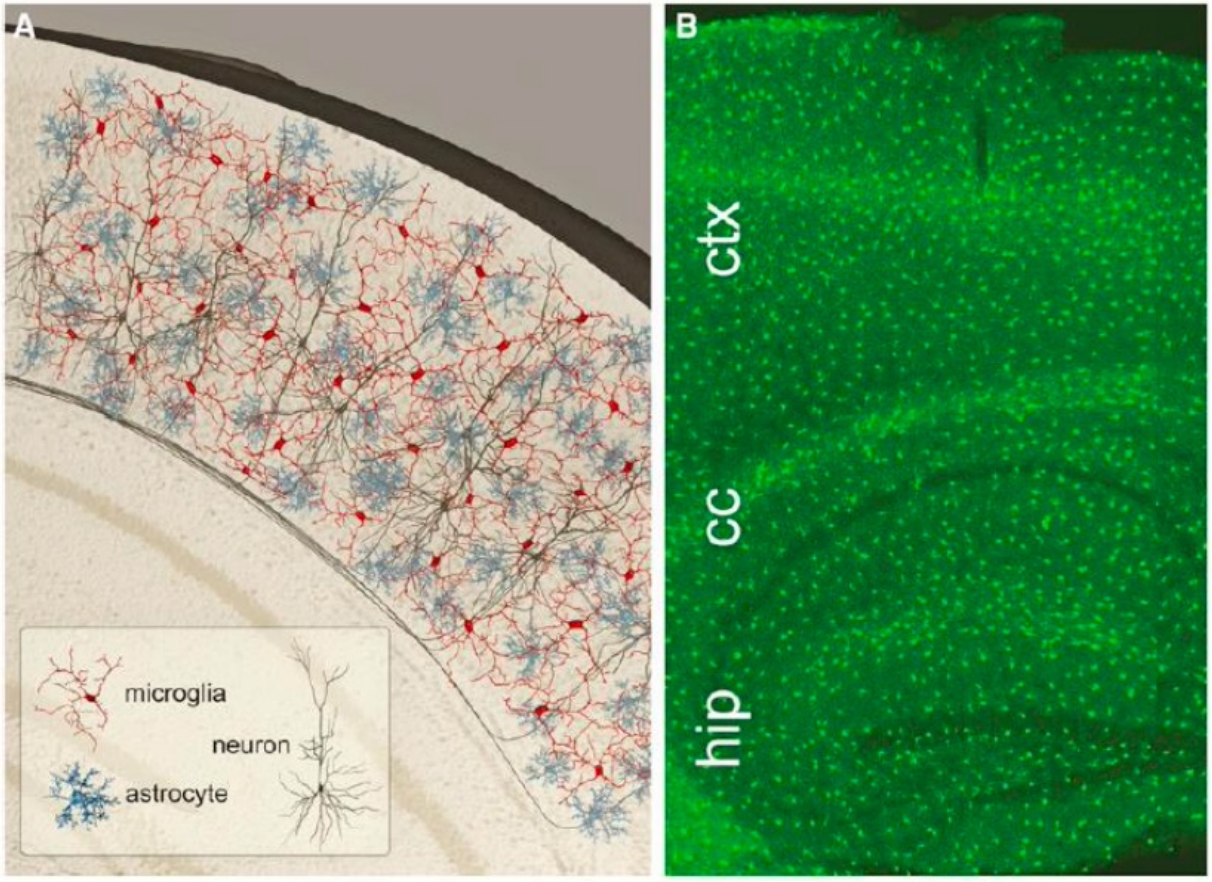


Figure 1. Uniform Distribution of Microglia in the Central Nervous System

(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).

Properties of microglial cells

- Major immunocompetent cells in the CNS

- Derive from monocytic lineage and migrate during early postnatal development

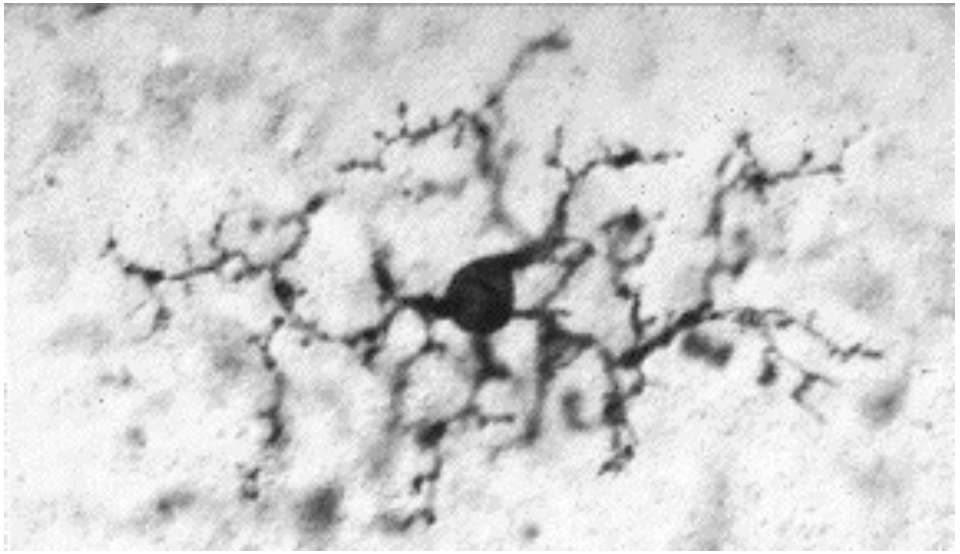
- Resting microglial cells (ramified) monitor brain environment and are activated in case of damage

- Activated microglia (ameboid) migrate to the site of injury, proliferate, release cytokines, have phagocytic activity

- Activated microglia show a distinct pattern of voltage-gated currents

Microglia act as phagocytes to protect brain from microorganisms

- Smallest cell bodies among the neuroglia.
- Main phagocytic cell
- Express neuroprotective proteins
- Antigen-presenting cells in the CNS, aiding in immune response (MHC-II)



Microglia (as opposed to Macroglia=astrocytes, oligos)

- Most like tissue macrophages elsewhere in body; not of neuroectodermal origin, like all macroglia
- Chief mediators of immune responses in brain
- CNS is not completely isolated from immune reactions
- Microglia derive from marrow monocyte lineage
- Have phenotypic markers similar to tissue macrophages:
- CD68, HAM-56, IL-1alpha,beta, class II MHC, OX-42

Origin of microglia and their colonization of the brain

Prenatal period: mesodermal progenitors

entry via meninges and ventricles

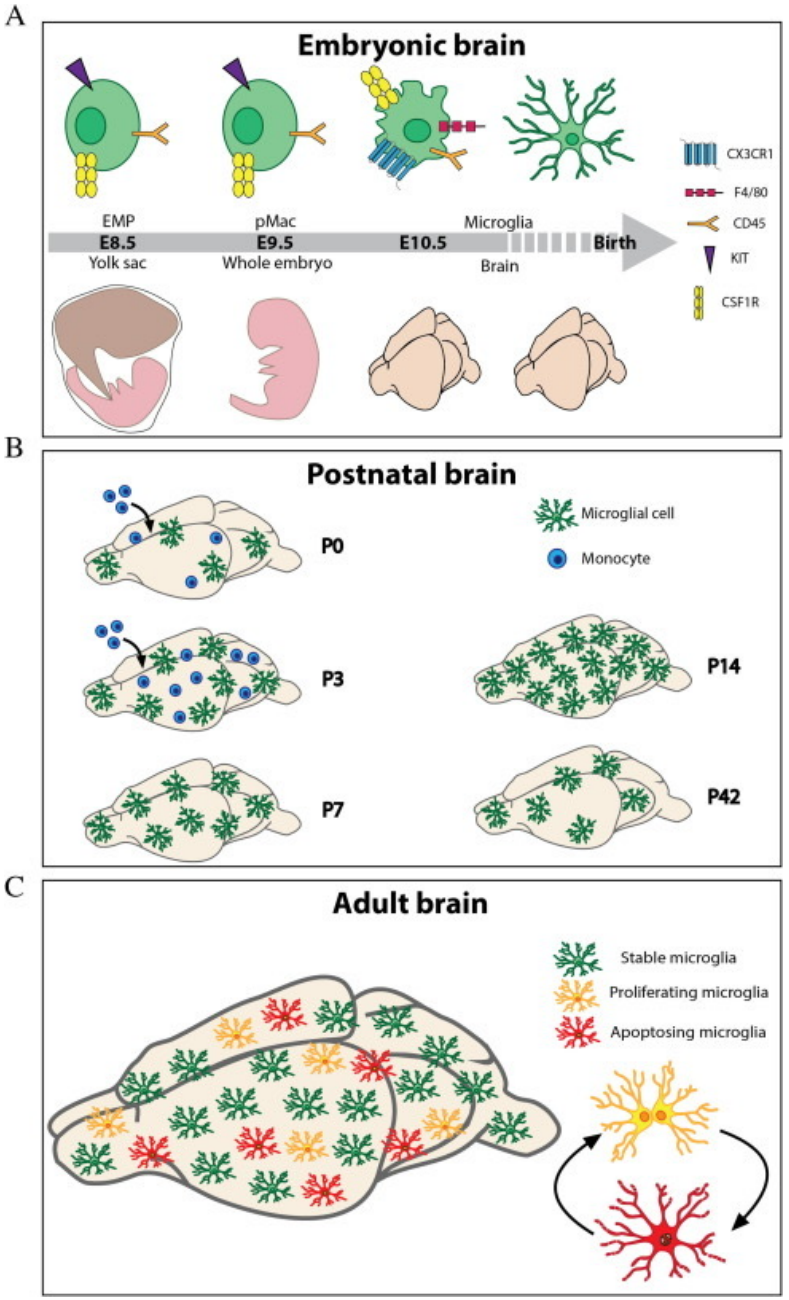
resident microglia in parenchyma

Postnatal period: circulating blood monocytes

entry via blood vessels

renewal of perivascular microglia

infiltration of parenchyma



Origin of microglia and their colonization of the brain

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.

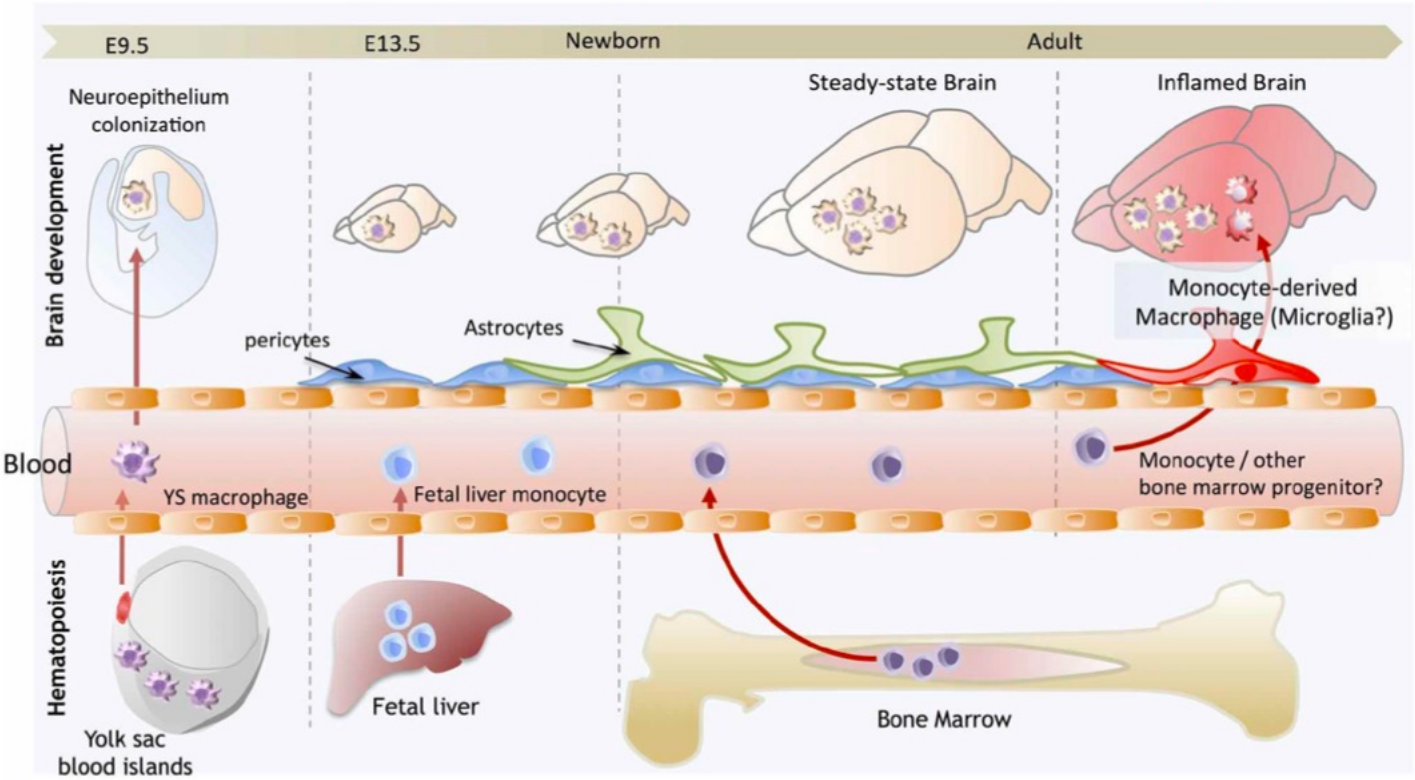
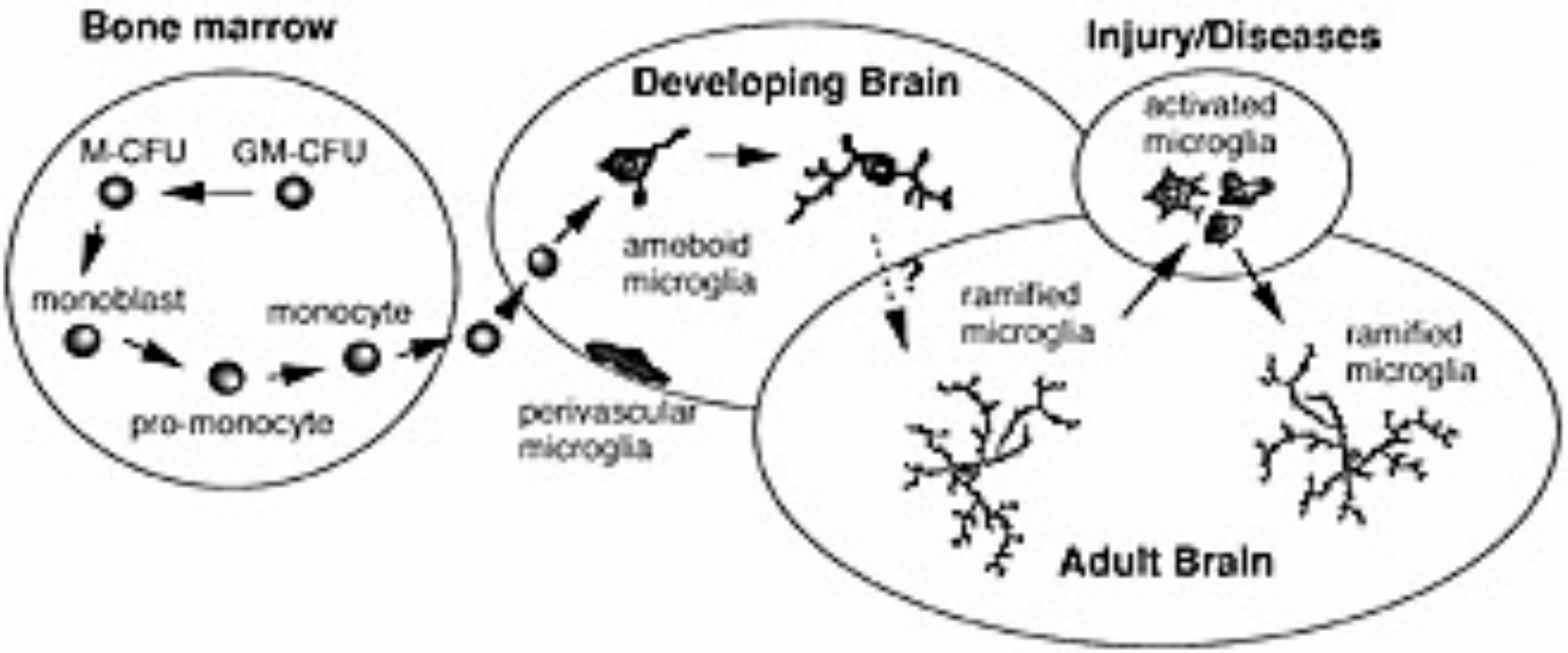


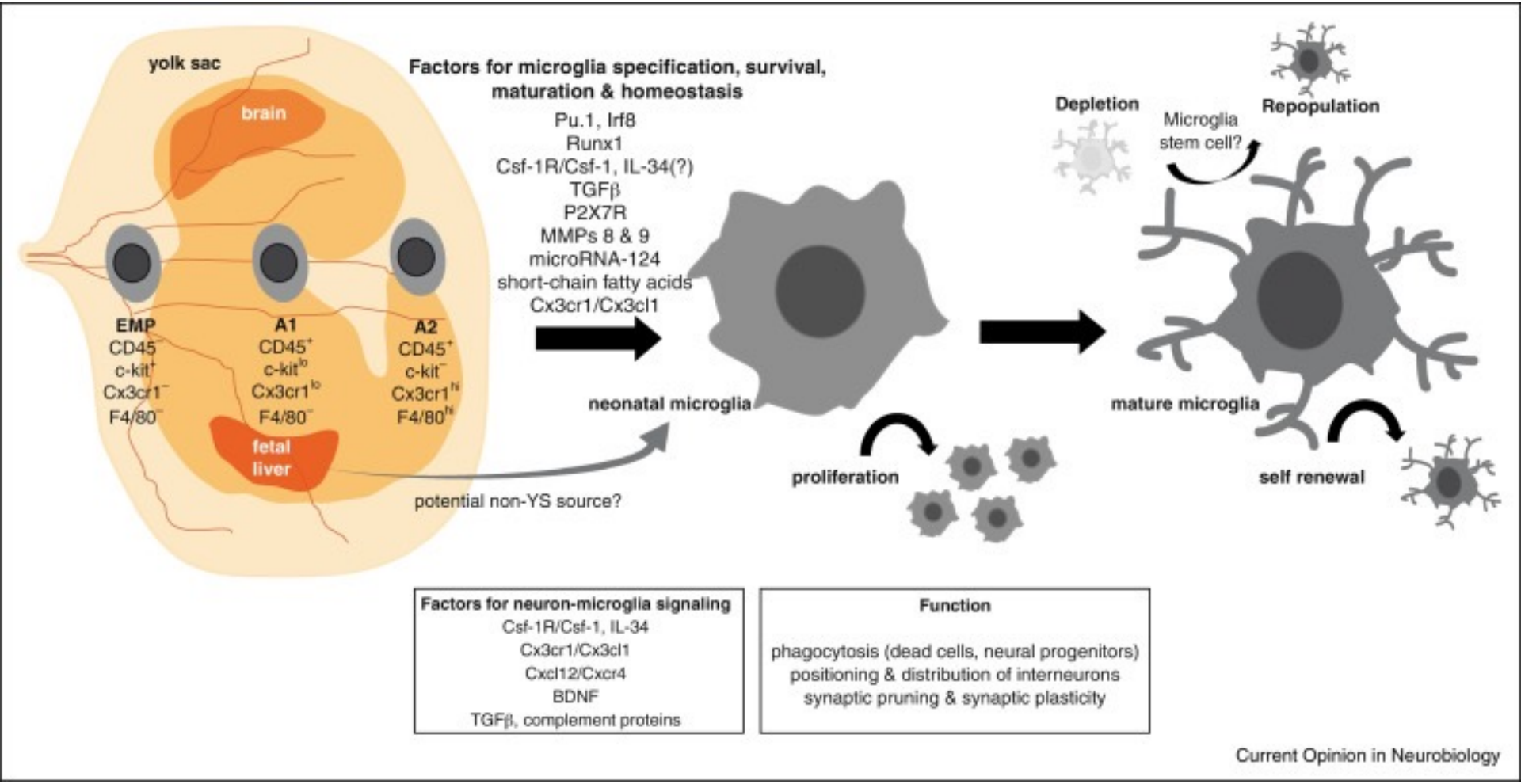
FIGURE 1 | Brain development and microglial homeostasis. Primitive macrophages exit the yolk sac blood islands at the onset of circulation and colonize the neuroepithelium from E9.5 to give rise to microglia. The blood brain barrier starts to form from E13.5 and may isolate the developing brain from 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

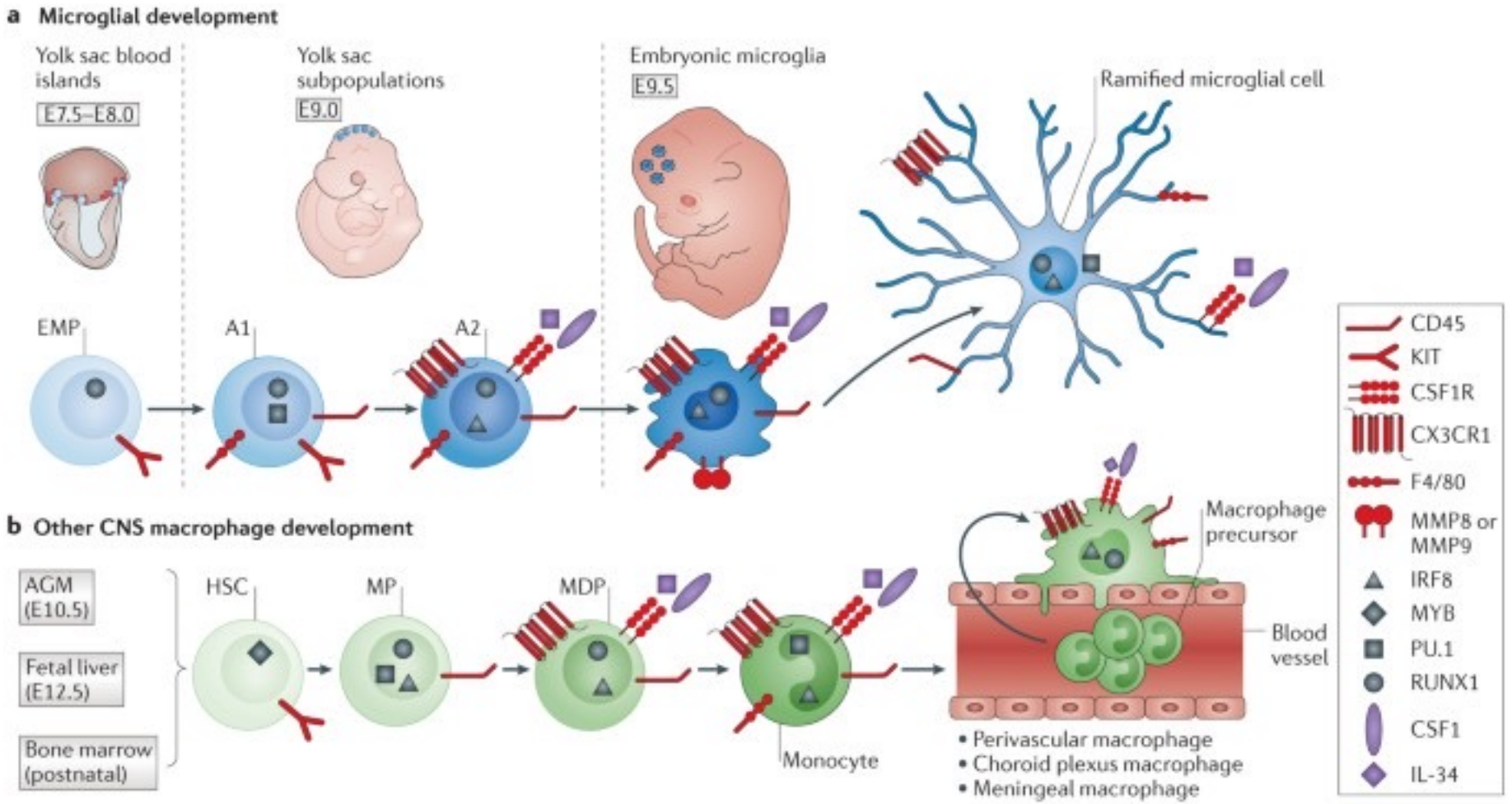
Microgliogenesis



Microgliogenesis



Microgliogenesis



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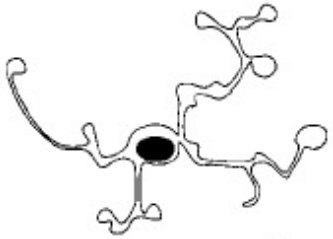
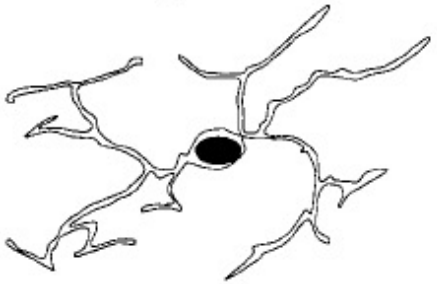
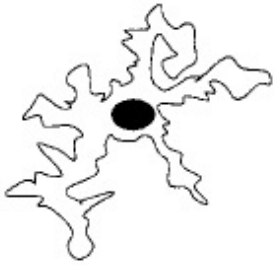
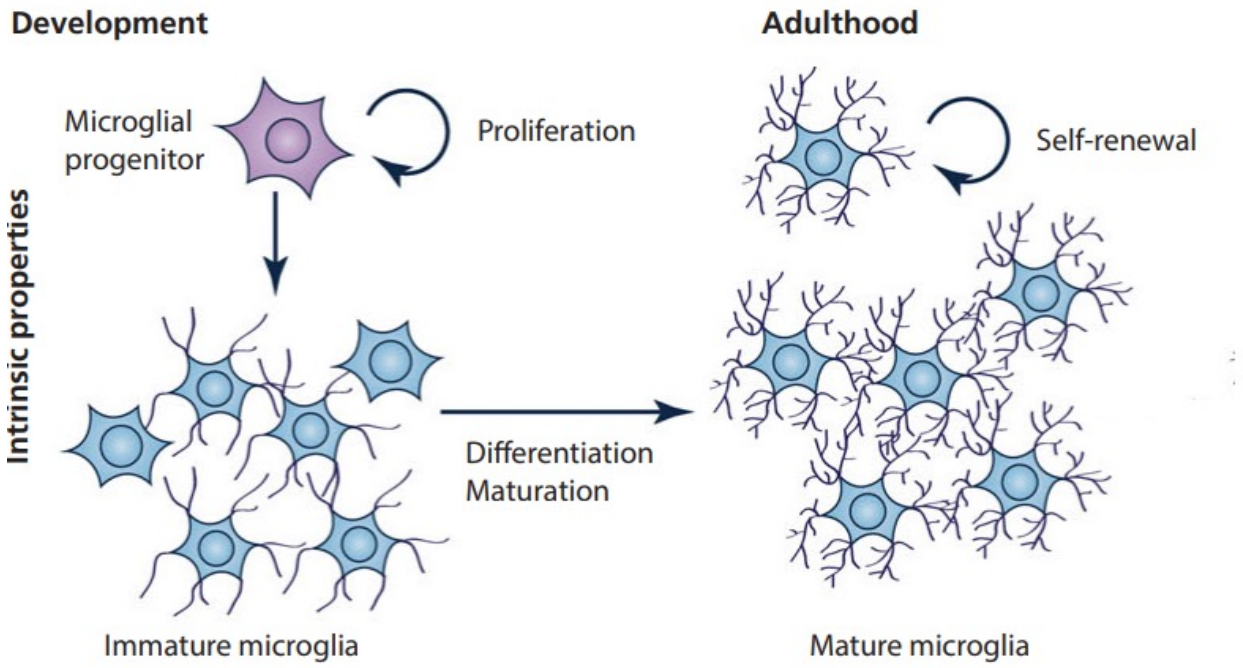
Microgliogenesis

Type of cell	Shape	Cell processes	Diameter	NDPase activity	Time course of appearance	Cell morphology
Ameboid microglia type 2 (1	Round	None, occasional filopodia	15-20 µm (2	High	P0-P9, scarcely at P12	
Ameboid microglia type 3 (1	Pleomorphic	Filopodia and/or Pseudopodia	15-50 µm (2	Moderate	P0-P9, some at P15	
Primitive ramified microglia (1	Oval to slightly elongated	Scantly developed processes showing a beaded shape	50-75/85 µm (3	Low	P0-P12, some at P15 and rarely at P18	
Resting microglia	Oval to roundish	Fully developed processes	85-100 µm (3	Low	Some at P12, P15-P18	
Reactive-like microglia	Large, plump, round to oval	Retracted, coarse processes	40/50-80 µm (3	Very high	Mainly from P9 to P18	

Microgliogenesis

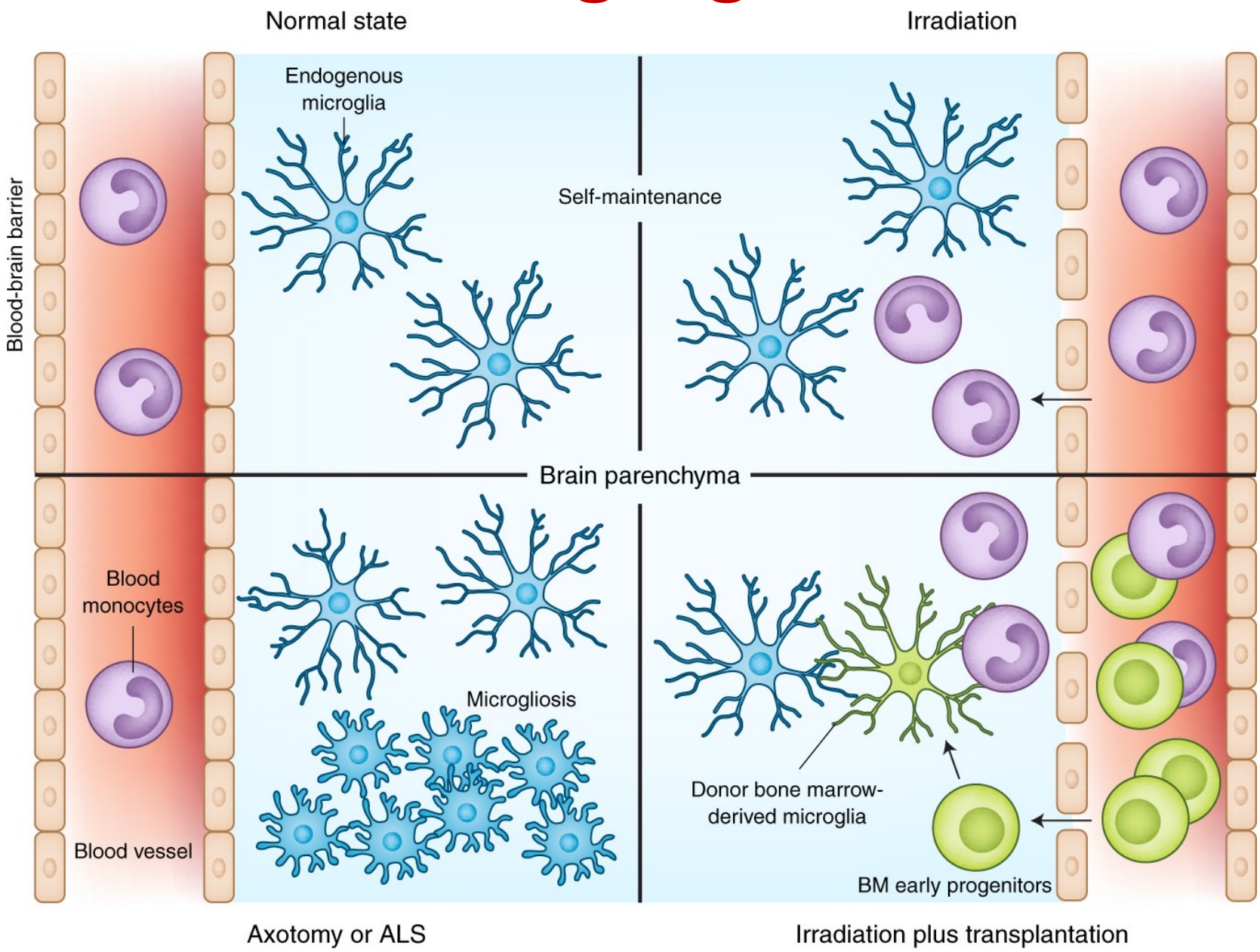


Leng Tai et al., 2016

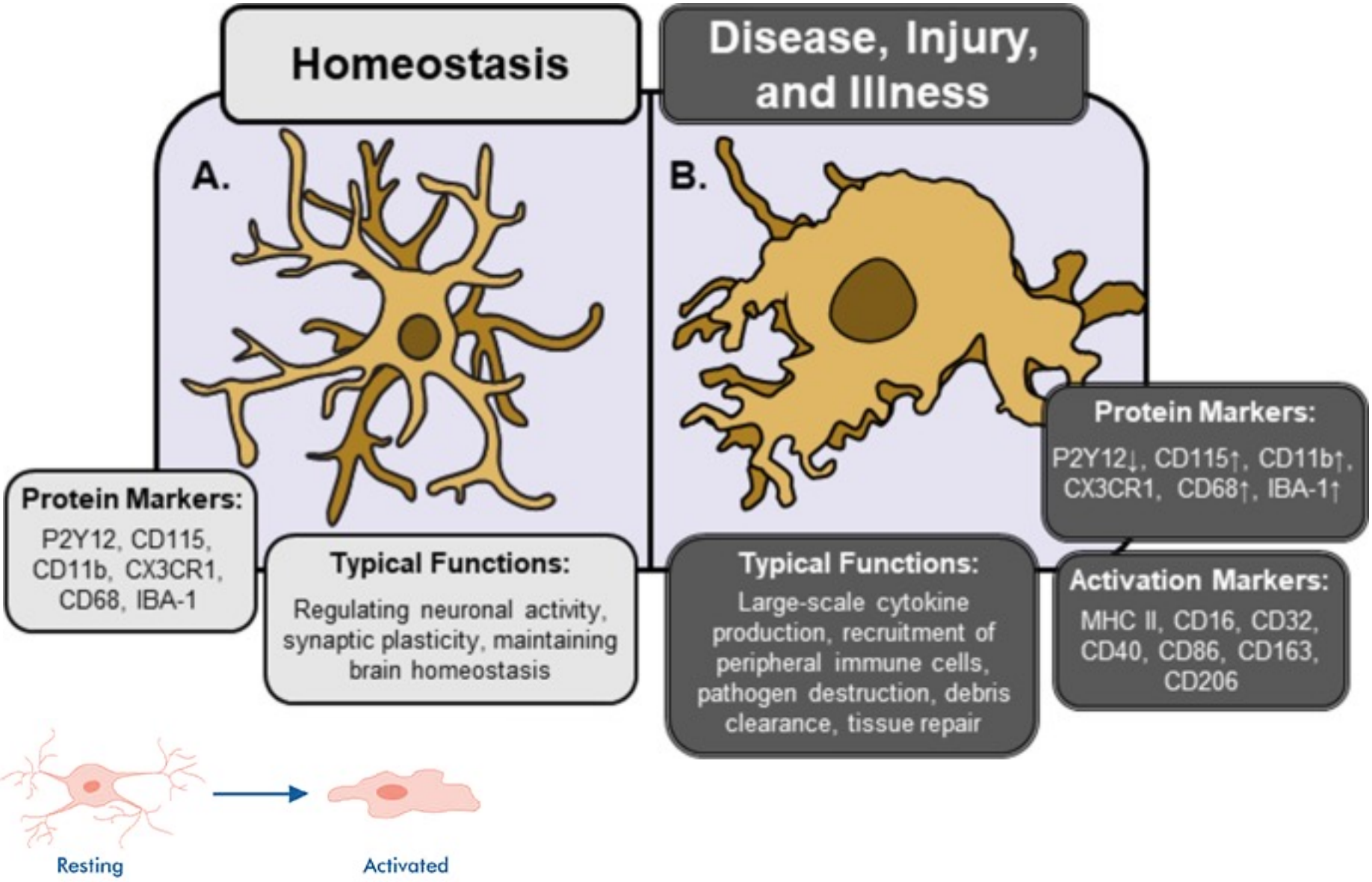
Early development: microglia progenitors are similar to macrophages, showing an ameboid morphology, which facilitates migration.

Late development: microglia get a mature morphology characterized by small cell bodies and longer, more ramified processes

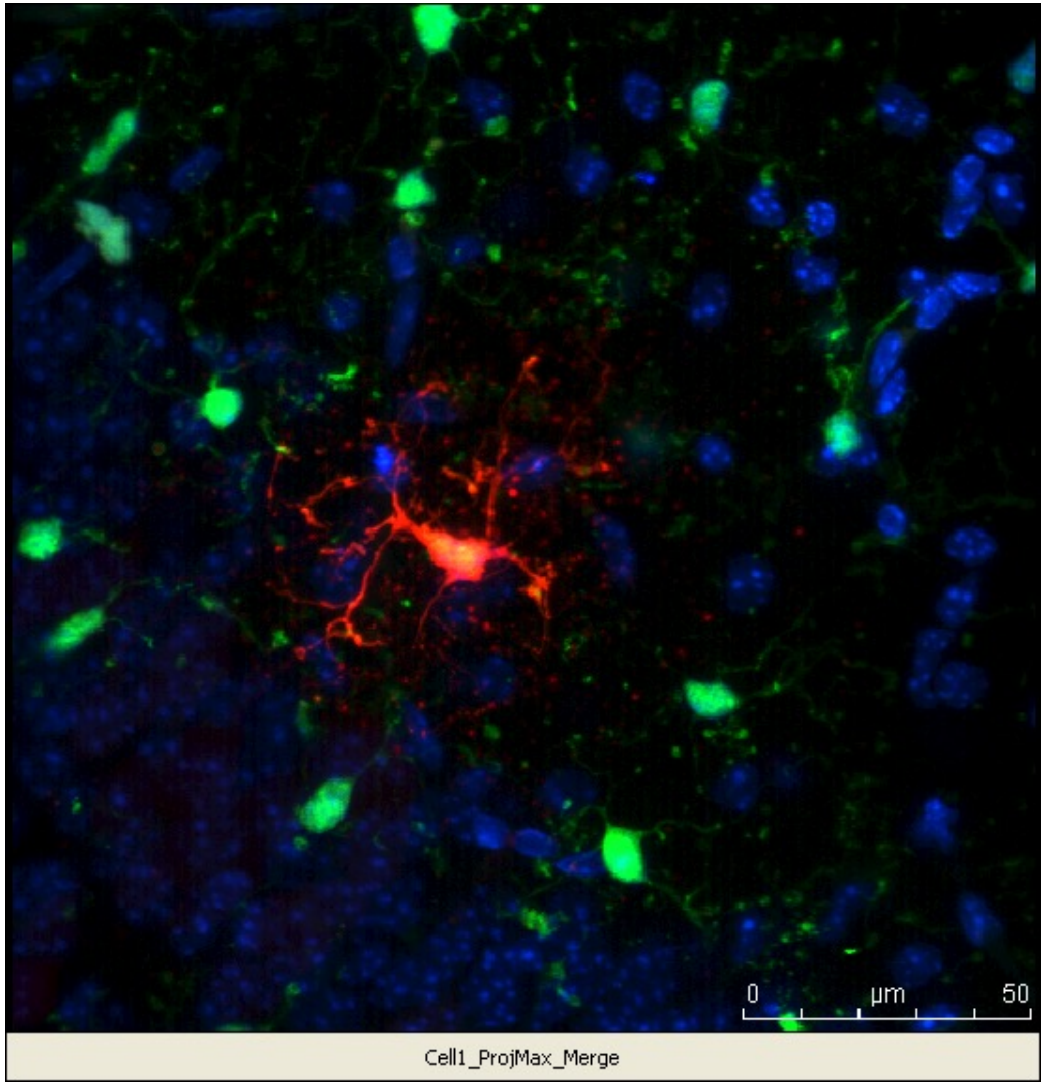
Microgliogenesis



Microglia activation… old view



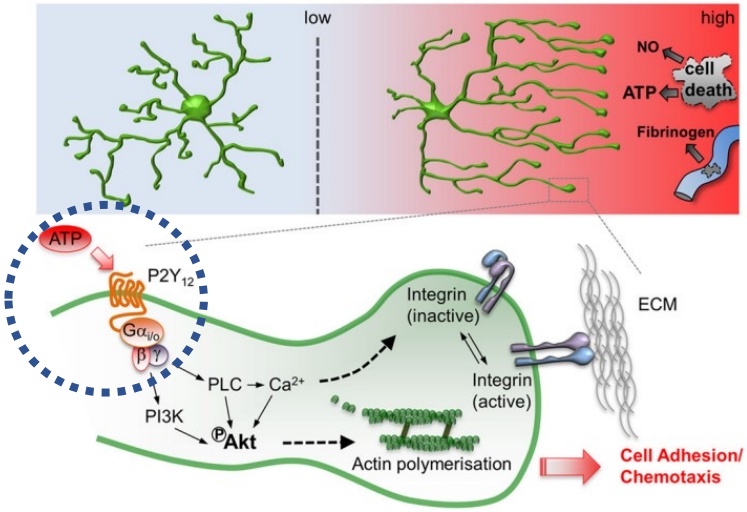
Never resting microglia



Never resting microglia

Resting Microglial Cells Are Highly Dynamic Surveillants of Brain Parenchyma in Vivo

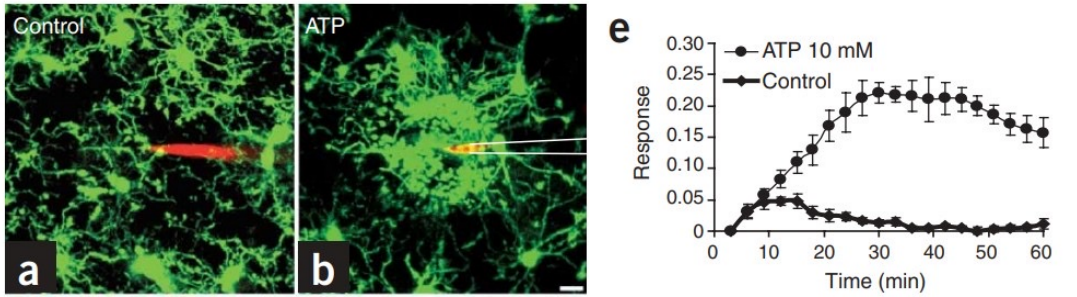
Axel Nimmerjahn,[1] Frank Kirchhoff,[2] Fritjof Helmchen[1]*



Madry et al., 2015

ATP mediates rapid microglial response to local brain injury *in vivo*

Dimitrios Davalos[1], Jaime Grutzendler[1,3], Guang Yang[1], Jiyun V Kim[2], Yi Zuo[1], Steffen Jung[2], Dan R Littman[2], Michael L Dustin[2] & Wen-Biao Gan[1]



Davalos et al., 2005

Microglia efficiently respond to ATP released from damaged cells via process rearrangement towards the injury site

Never resting microglia

Microglia constantly move their processes to scan the brain parenchyma

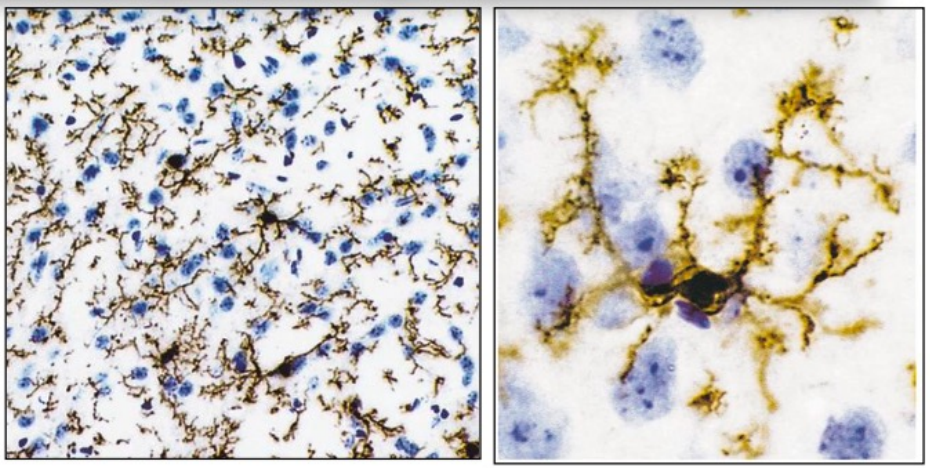


Figure 4–19 Large numbers of microglia reside in the mammalian central nervous system. The micrograph on the left shows microglia in the cerebral cortex of an adult mouse (in **brown**, immunocytochemistry). The **blue** spots are the nuclei of nonmicroglial cells. The microglial cells have fine, lacy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

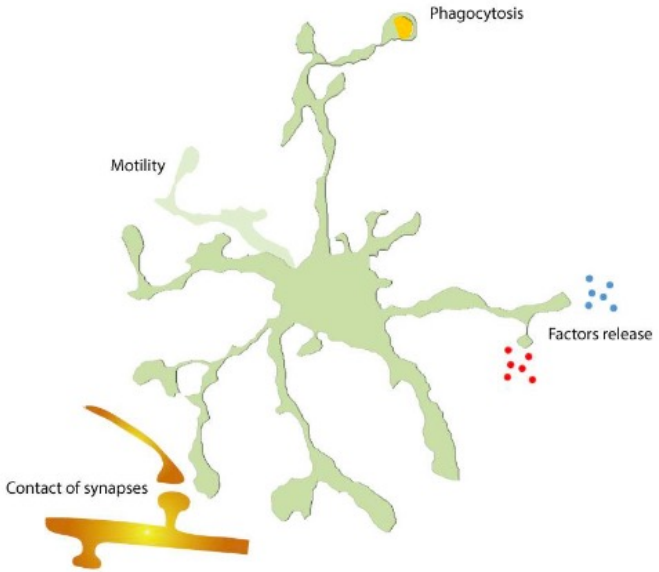


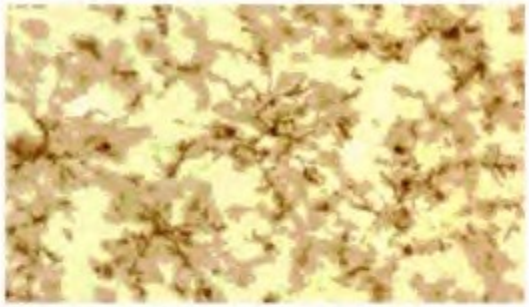
Figure 2.6 Scheme of the different functions of microglia.
Microglia (green) constantly move their processes to scan the brain parenchyma. During their movements they contact synapses and neuronal dendrites (orange), as well other brain cells. They can control brain activity and surrounding cells' fate 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.

Microglia: sensors of changes in the CNS

Highly dynamic cells:
Typical morphological changes upon activation

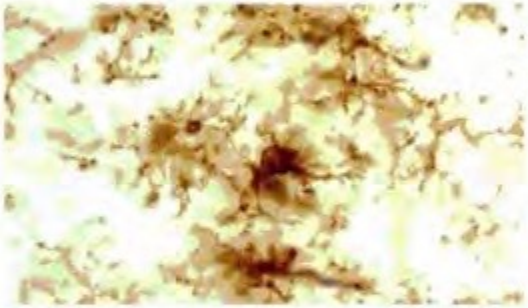
Resting Microglia
Stratum radiatum
CA1

Normal rat



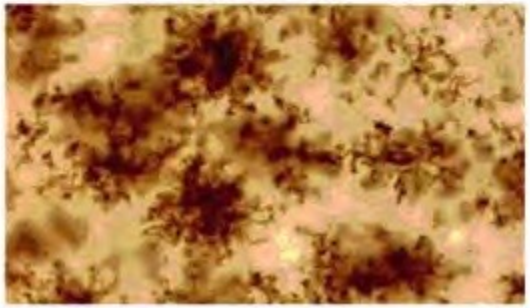
Early Activated Microglia
Stratum radiatum
CA1

One day after
4-vessel occlusion



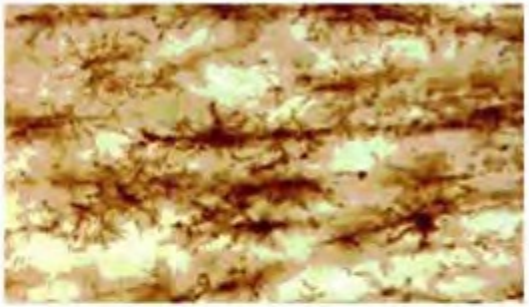
Bushy Microglia
Stratum radiatum
CA1

Four days after i.c.v.
kainic acid injection



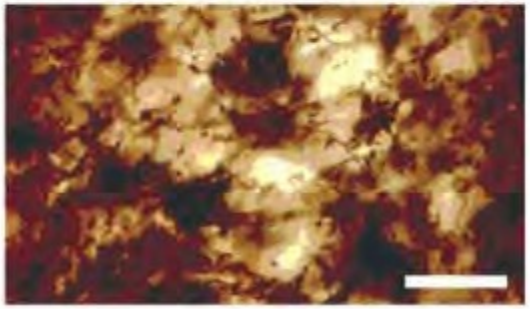
Microglial Rod Cells
Stratum radiatum
CA1

Seven days after
4-vessel occlusion



Brain Macrophages
Stratum pyramidale
and radiatum CA3

Four days after i.c.v.
kainic acid injection



Microglia: sensors of changes in the CNS

Morphological change following microglial activation

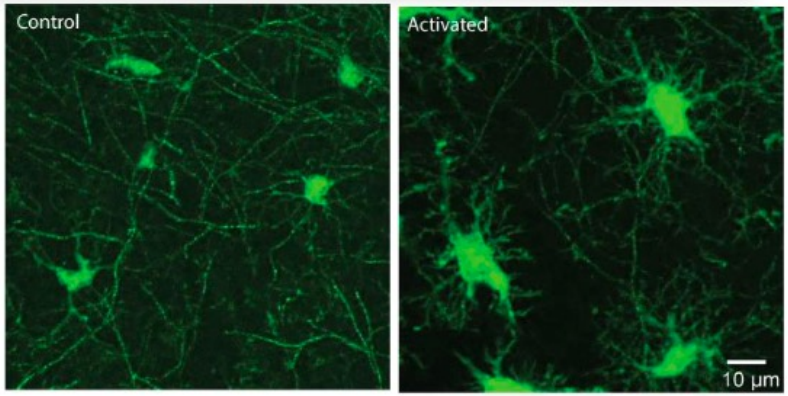


Figure 2.7 Microglia change properties after activation. The images show an example of morphological changes of microglia 48 hours after activation induced by *status epilepticus*. In control conditions (**a**) microglial cells have a small body with long and ramified processes. (**b**) In contrast, 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

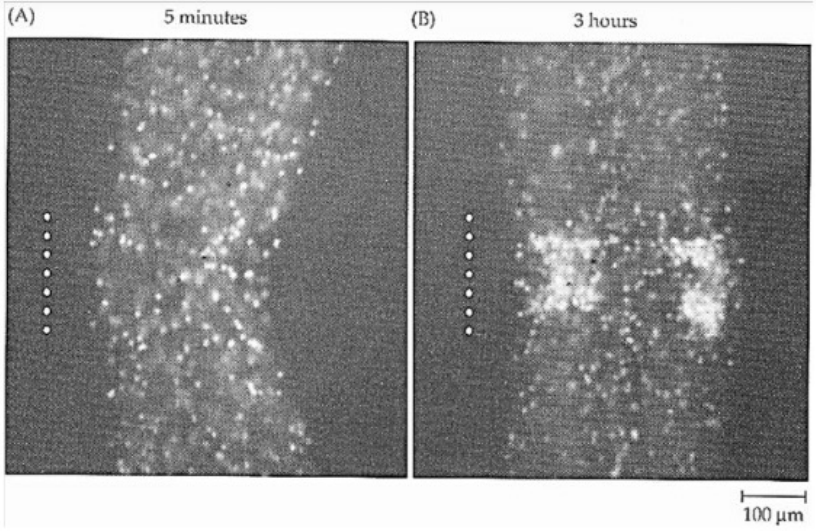
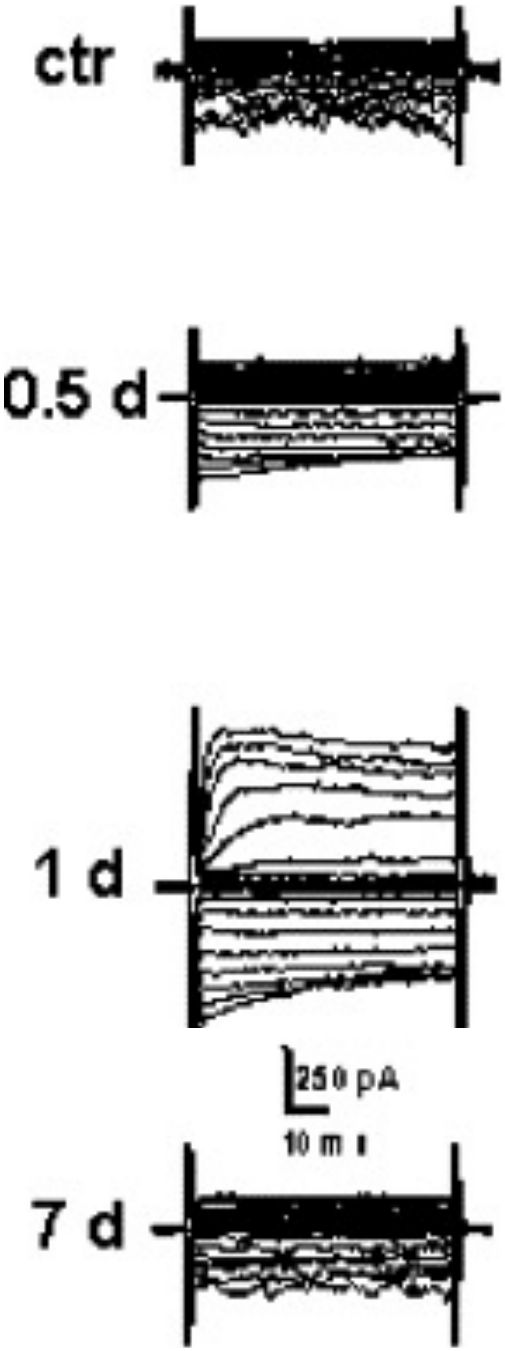


FIGURE 8.11 Migration of Microglial Cells in Injured CNS. (A) Microglia in the leech CNS were stained with a fluorescent nuclear dye (Hoechst 33342). The bundle of axons linking ganglia had been crushed 5 minutes earlier. The extent of the crush is indicated by the dotted line. The nuclei of microglial 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-

K^+ currents of microglial cells.

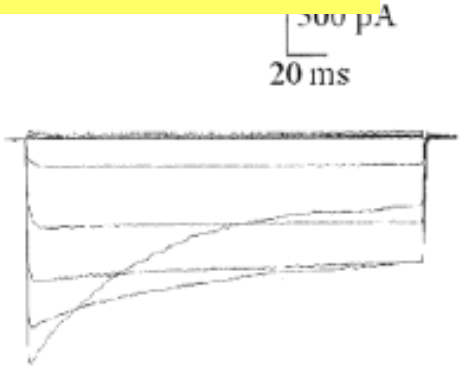


Ramified microglial cells from acute brain slice of unlesioned brain are physiologically distinct from cultured microglial cells. Membrane current of resting microglia in situ (ctr) and current pattern after facial nerve axotomy (left, 0.5 to 7 days),

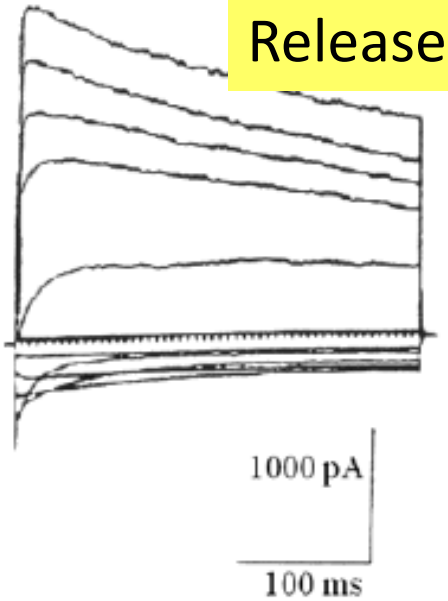
- Inward rectifying K^+ currents preceed delayed rectifying
- Activation correlates with resting potential hyperpolarization

Voltage activated ionic channels in microglial cells

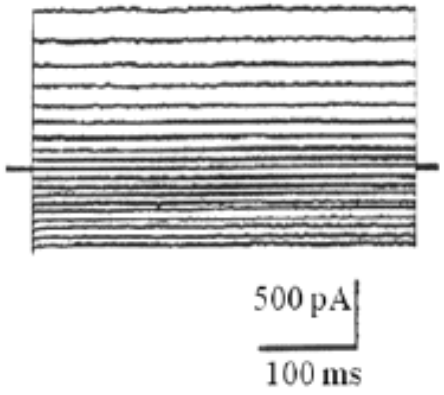
Proliferation
Resting potential



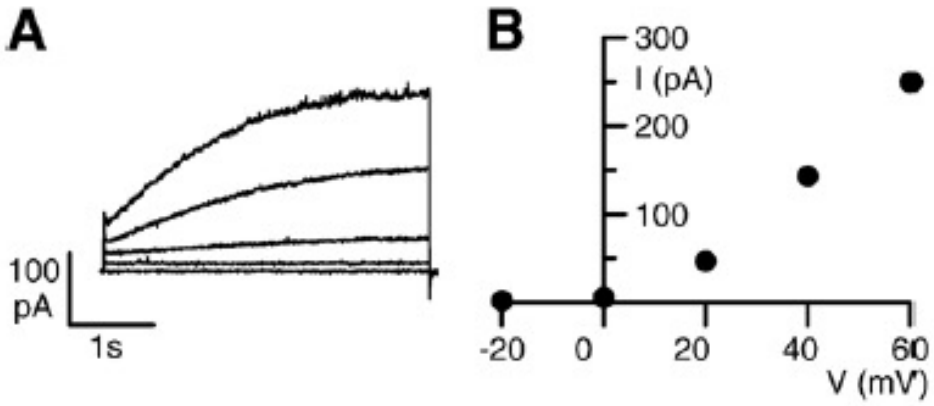
Proliferation
Release of IL-1b



Ramification



inward rectifier K^+ outward rectifier K^+ Stretch-activated Cl^-



Release of ROS

Proton current H^+

Microglia in the adult brain

Pathogen
Cell debris
Myelin debris

Microglia activation
(phagocytosis, release of ROS, cytokines, chemokines)

Protective or destructive

Microglia in the adult brain

- Resident macrophage population of the CNS

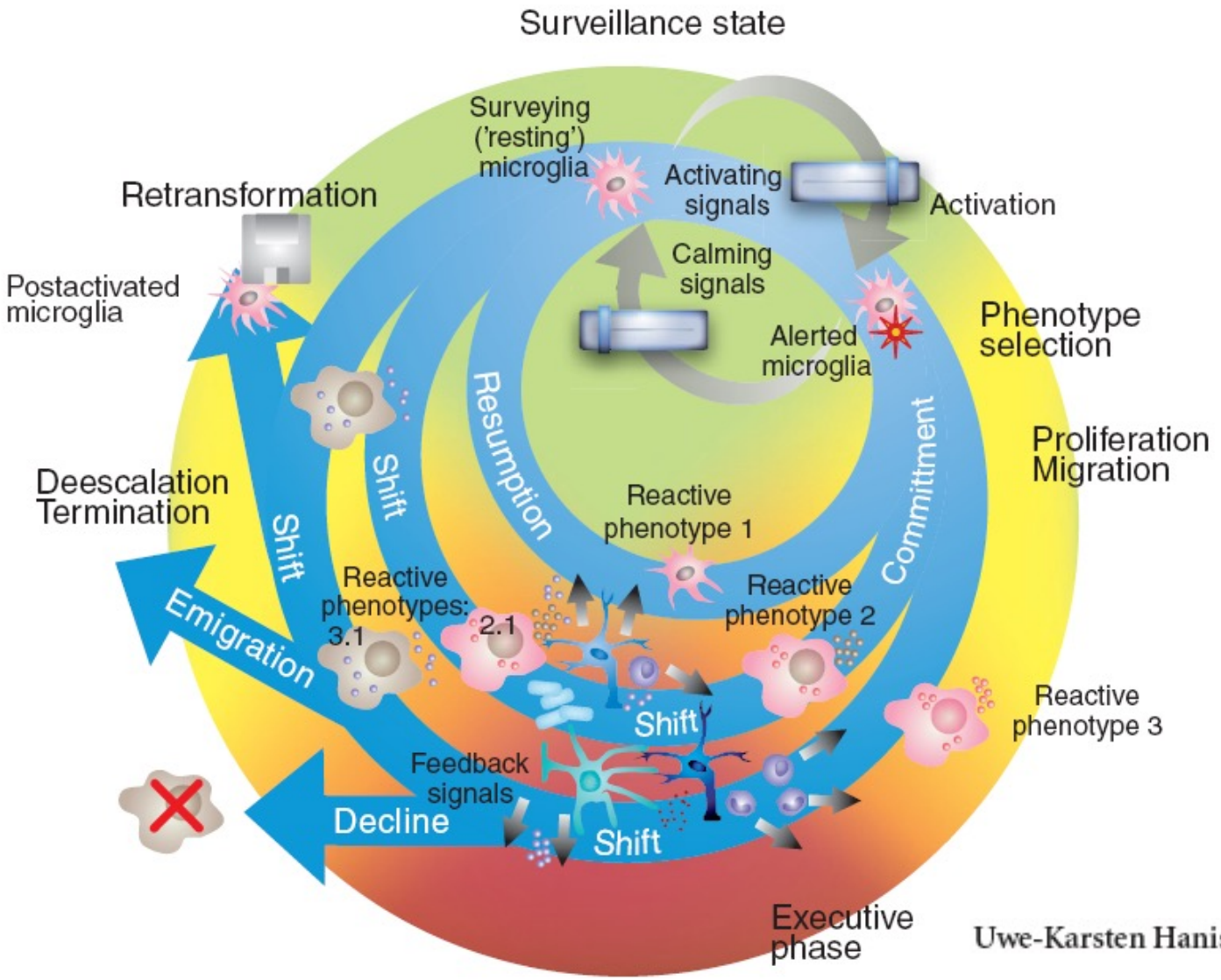
- **Active tissue scanning**

- Transformation of microglia to reactive states in response to pathology

- **Engagement of microglia can be either neuroprotective or neurotoxic, resulting in containment or aggravation of disease progression**

- **Microglial responses in different pathologic contexts**

Microglia activation - now



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nature neuroscience
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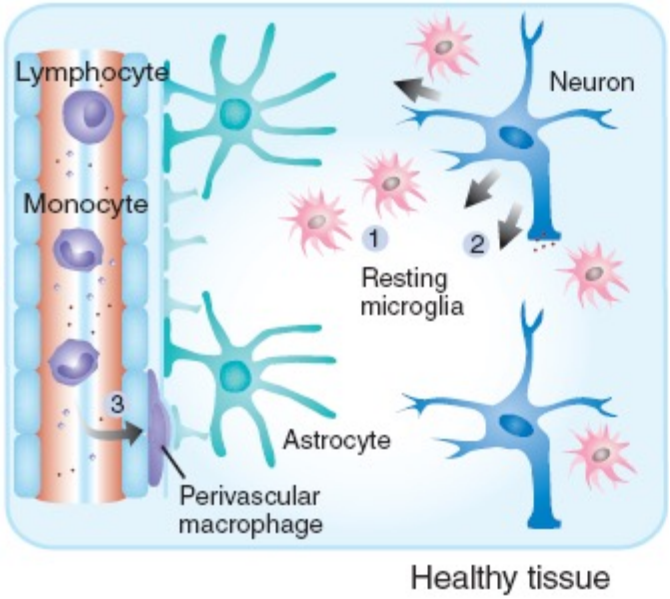
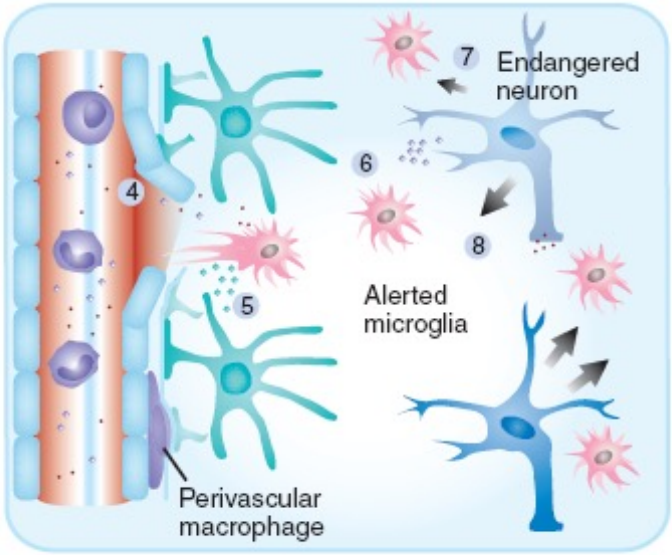
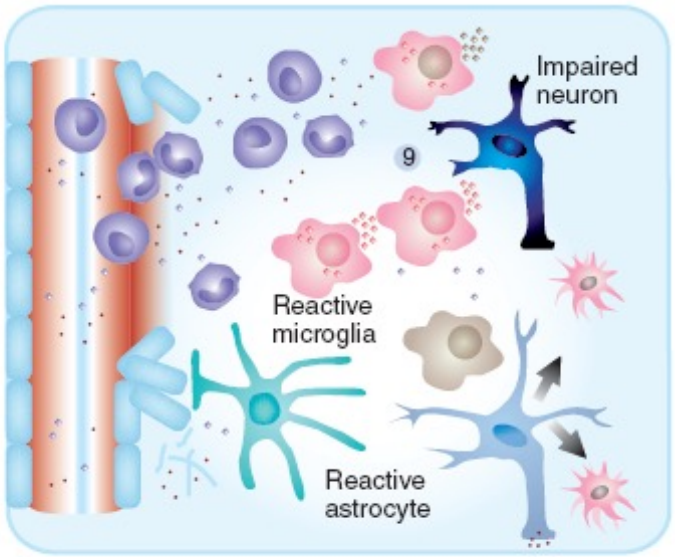
Microglia activation



Healthy tissue



Small local damage



Large insult

Uwe-Karsten Hanisch & Helmut Kettenmann

nature neuroscience

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Microglia activation - now

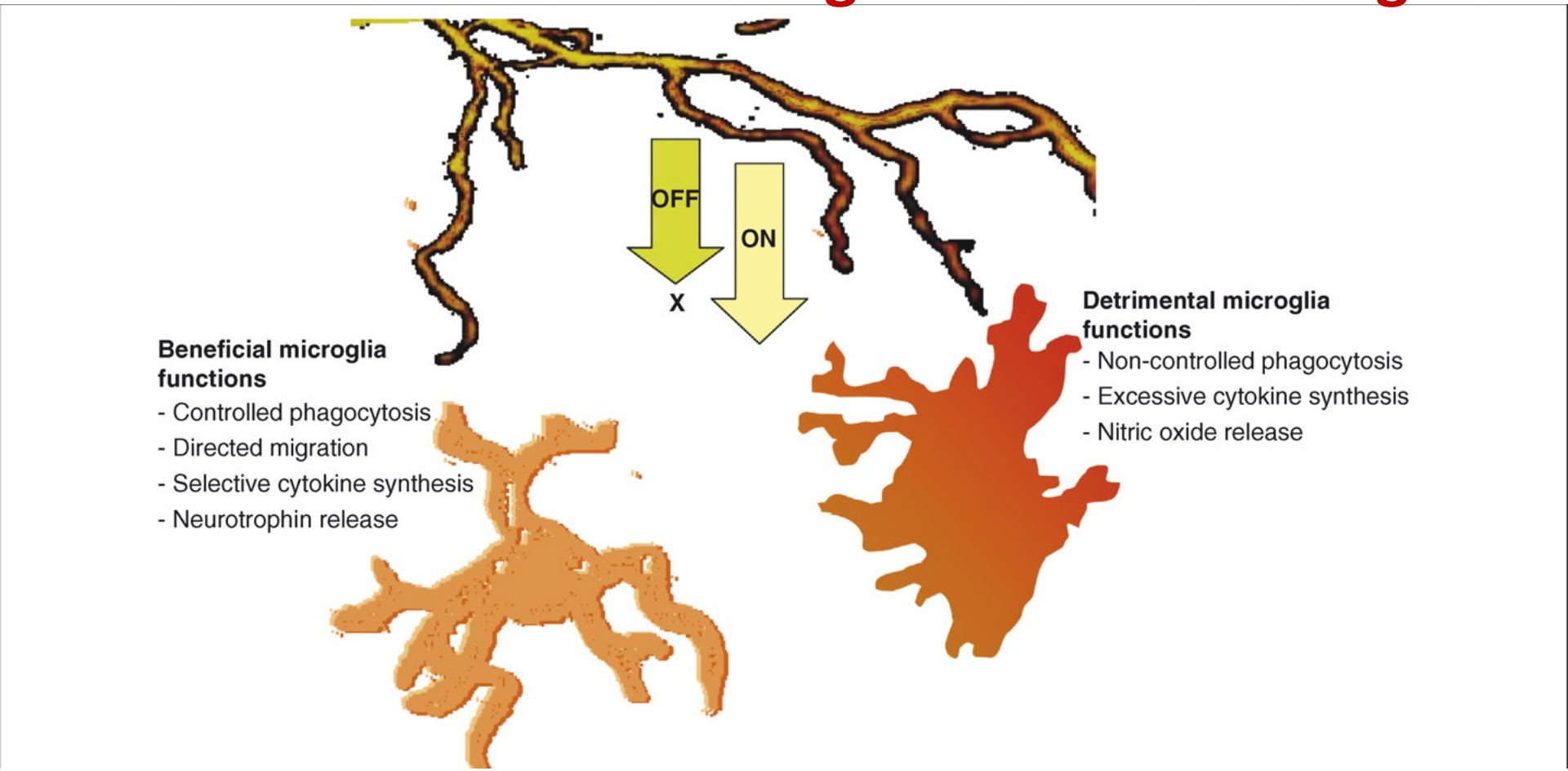
Microglial activity states throughout the activation process. Microglial cells in the surveillance constantly scan for signals of potential threat to CNS homeostasis.

1.The appearance of such ‘activating’ signals (infection, trauma) or the loss of constitutive ‘calming’ signals triggers a transition to an alerted state.

2. Further committment to distinct reactive phenotypes and executive phase (release of cytokines and chemokines, phagocytotic activity).

3.The reactive behavior of microglia is controlled by a fading (or elimination) of the initial activating signals as well as influences from resident CNS and invading immune cells

Neuronal ‘On’ and ‘Off’ signals control microglia



Off signals constitutive keep microglia in their resting state and antagonize proinflammatory activity.

On signals inducible (purines, chemokines, glutamate) instruct microglia activation under pathological conditions towards a beneficial or detrimental phenotype

Microglia functional roles

How many functional roles of MICROGLIA?

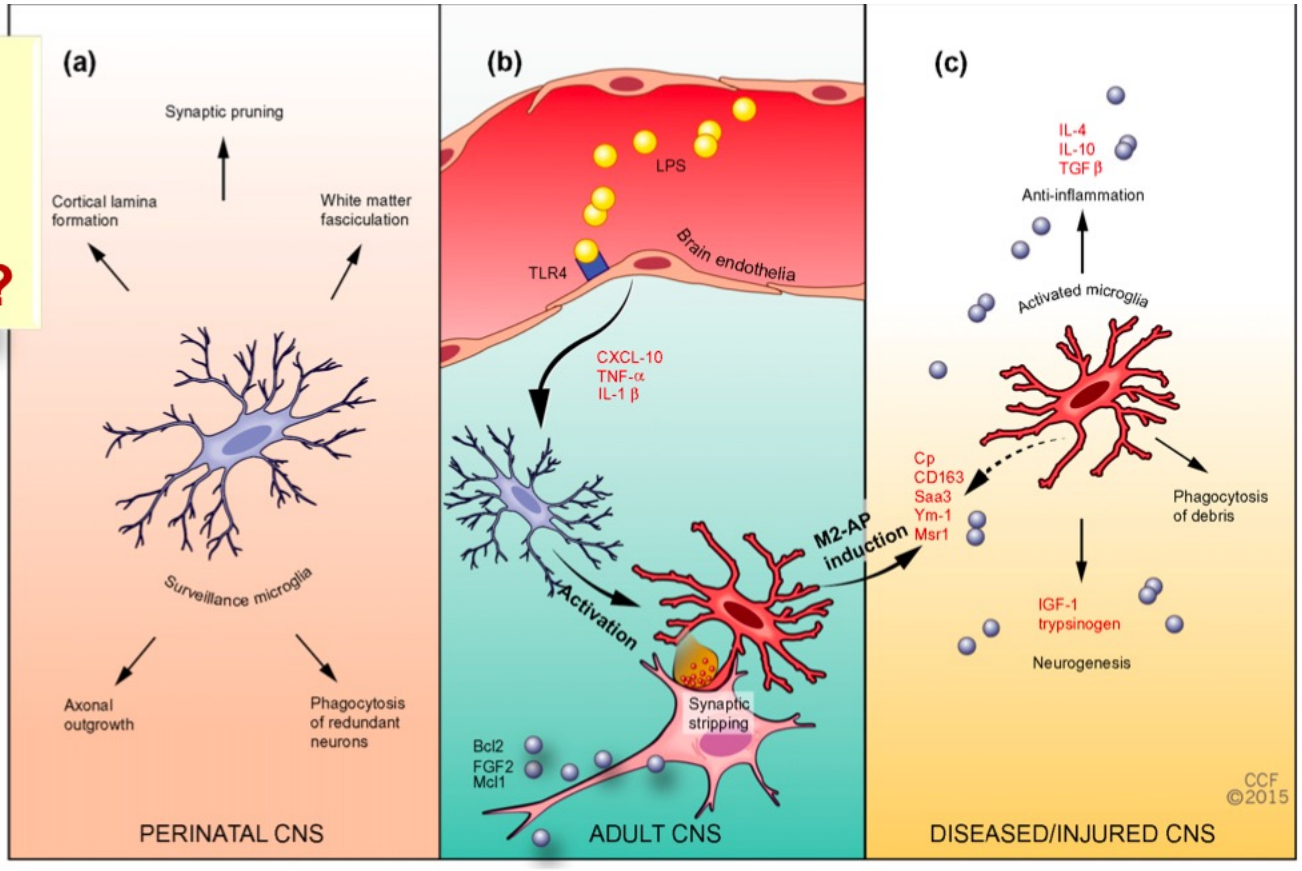


Fig. 1 Microglia contribute to CNS homeostasis and neuroprotection during development (a), adulthood (b), and CNS diseases (c). (a) Microglia maintain tissue homeostasis during brain development by pruning synapses or phagocytizing redundant neurons. They also participate in the proper formation of CNS structures, including cortical lamina formation and axon bundle fasciculation. (b) Peripherally delivered LPS can activate TLR4 receptors on the luminal surface of brain endothelial cells, which secrete cytokines to subsequently activated microglia. Activated microglia strip axosomatic inhibitory synapses from neuronal soma, which induces neuroprotection by up-regulating neuronal production of anti-apoptotic molecules such as Bcl1, FGF2 or Mcl1. In addition, these microglia can assume an M2-AP phenotype, which reduce oxidative stress in the event of an attack by secreting Ceruloplasmin (Cp), CD163, Saa3, Ym-1, and Msr1. (c) During CNS injury or in neurodegenerative diseases, microglia offer neuroprotection by producing anti-inflammatory cytokines, phagocytizing cellular debris, and promoting neurogenesis through production of IGF-1 or trypsinogen. They may also produce M2-AP proteins in fighting against oxidative stress (indicated by dotted arrow). LPS: lipopolysaccharide; TLR4: Toll-like receptor 4; CXCL10: C-X-C motif chemokine 10; Bcl2: B-cell lymphoma 2; FGF2: fibroblast growth factor 2; Mcl1: myeloid cell leukemia 1; AP: acute phase; Saa: serum amyloid protein; Ym-1: chitinase 3-like-3; Msr1: macrophage scavenger receptor 1; IGF: insulin-like growth factor.

Chen, 2016, J Neurochemistry
doi: 10.1111/jnc.13062

Microglia phagocytosis

Removal of apoptotic cells by phagocytosis

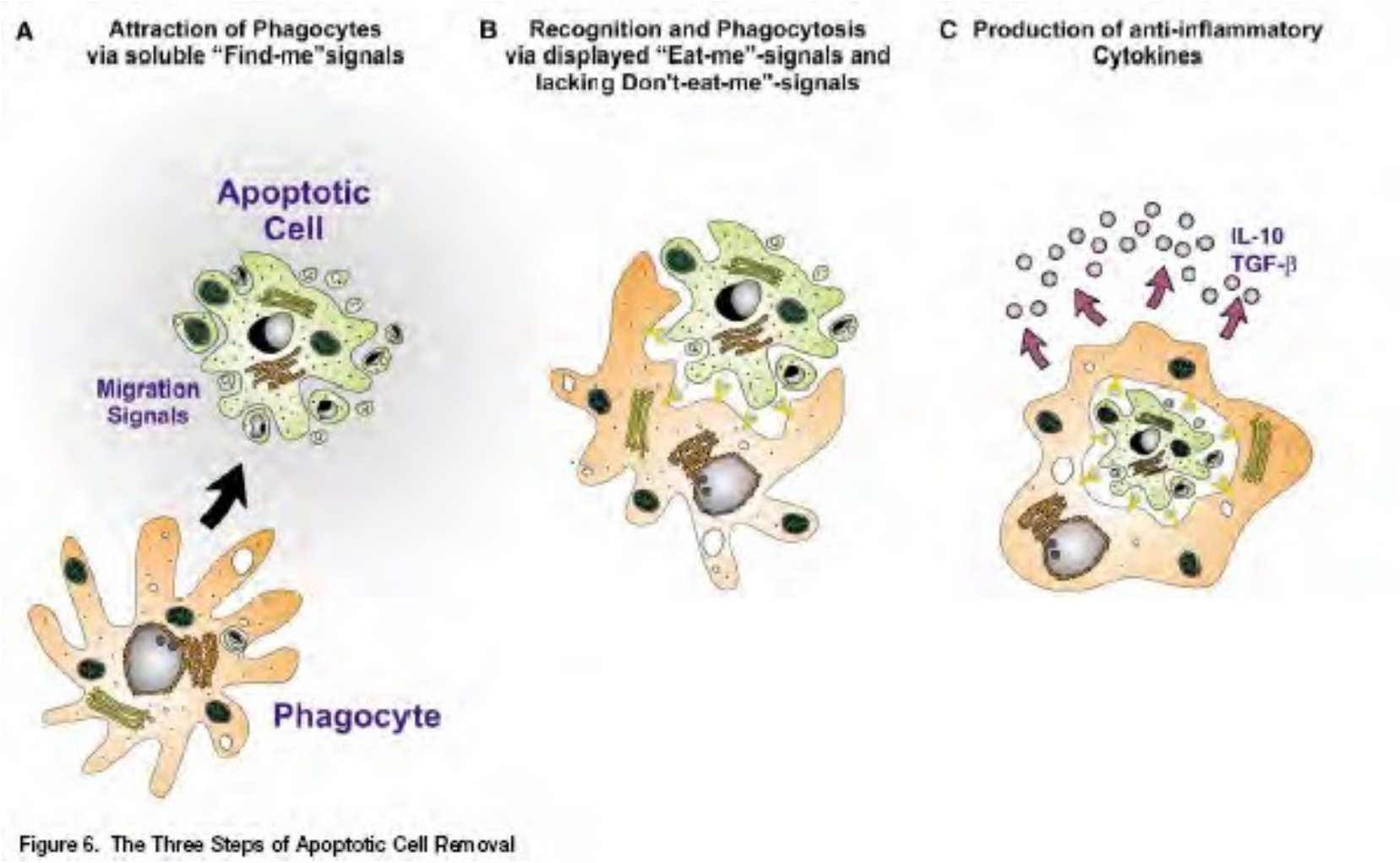
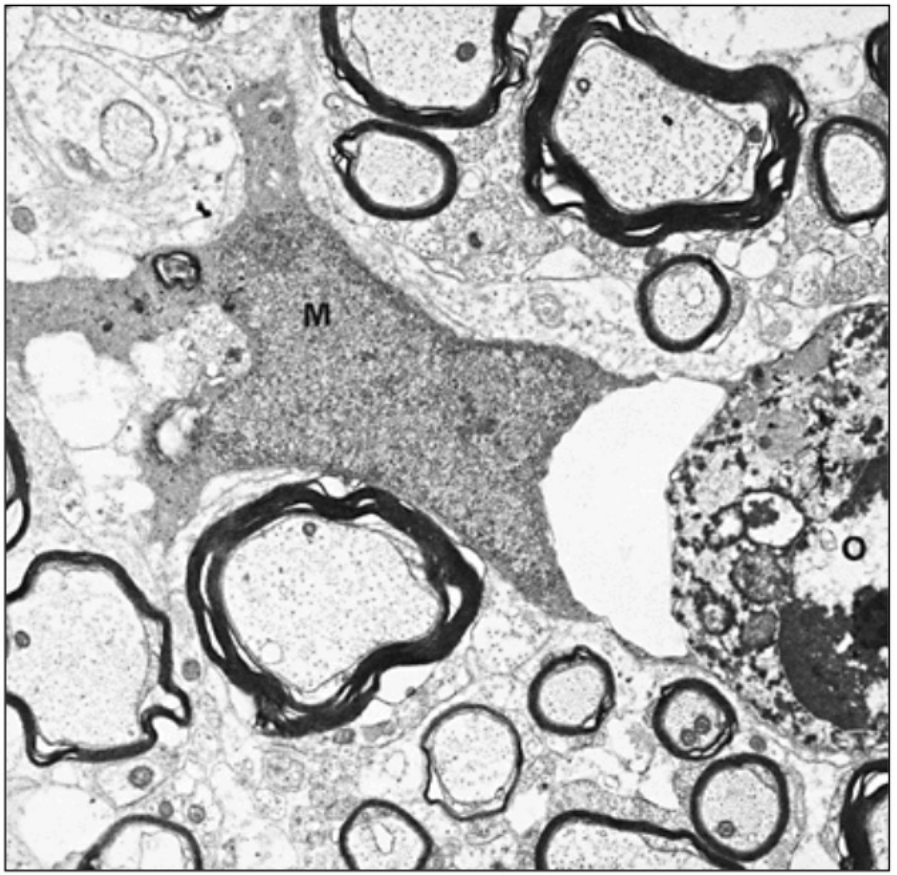


Figure 6. The Three Steps of Apoptotic Cell Removal

Microglial phagocytosis

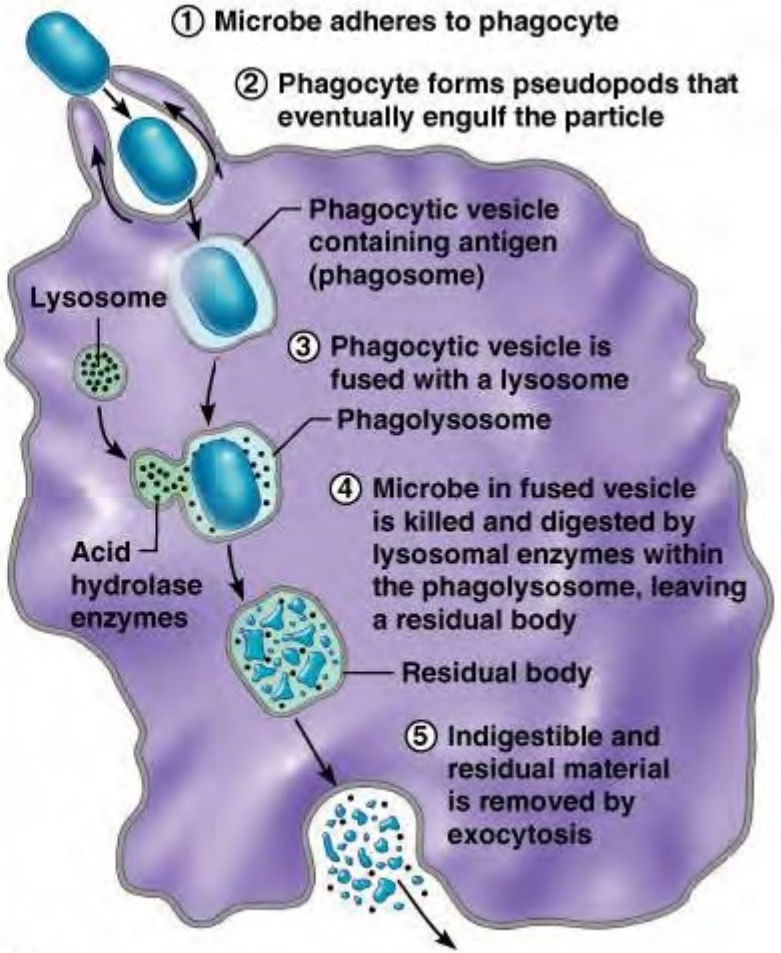
Phagocytotic activity of microglia

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 phagocytosis

(a)



(b)

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 30 January 2013
doi: 10.3389/fncel.2013.00006

Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis

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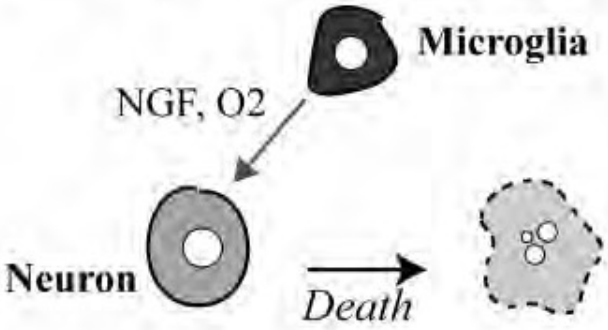
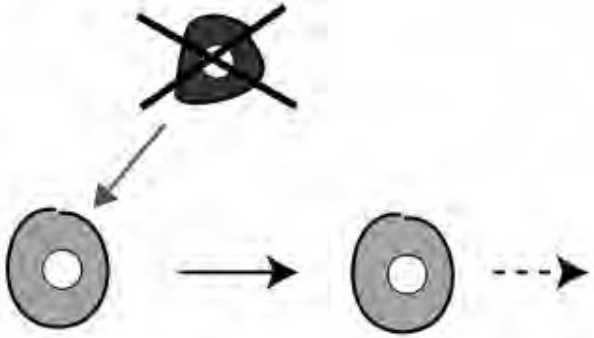
Microglia role in cell death

A. Microglia instruct developmental death (Cerebellum, retina).

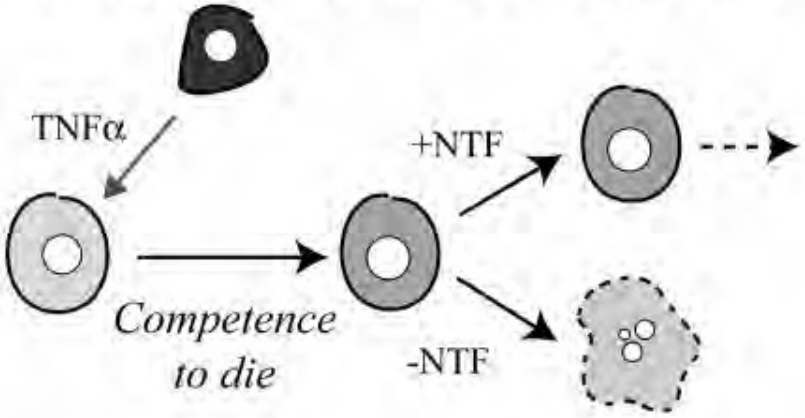


Microglia can induce developmental apoptosis.

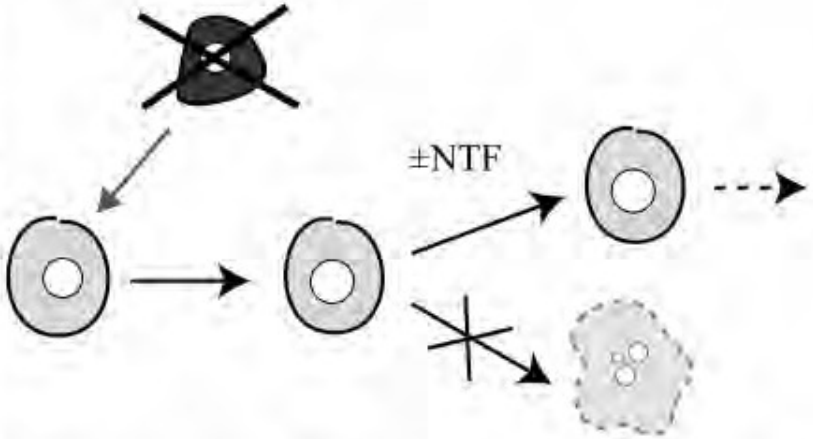


When microglia are removed or killed, no apoptosis occurs.

B. Microglia instruct motoneurons for a delayed sensibility to neurotrophic factors (Spinal cord).

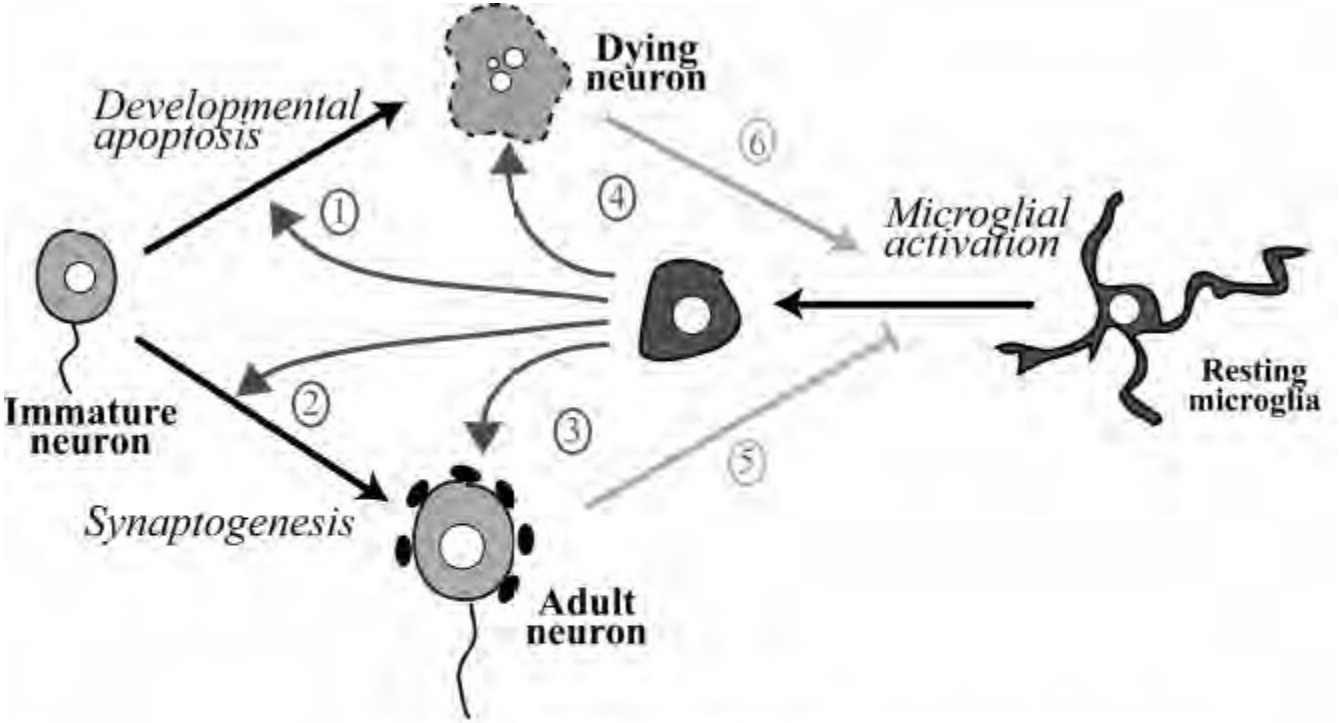


Motoneurons become sensitive to neurotrophic factors (NTF) and competent to die after instruction by microglial TNFα



When microglia are killed, motoneurons are no more sensitive to neurotrophic factor (NTF).

Microglia role in cell death



Microglia to neurons	1: Microglia control developmental apoptosis. 2: Microglial control synaptic development. 3: Microglia regulate synaptic properties. 4: Microglia engulf dead neurons.
Neuron to microglia	5: Neuronal activity inhibits microglial activation. 6: Damaged neurons induce microglial activation.

Microglia role in neurogenesis

Microglia and hippocampal neurogenesis

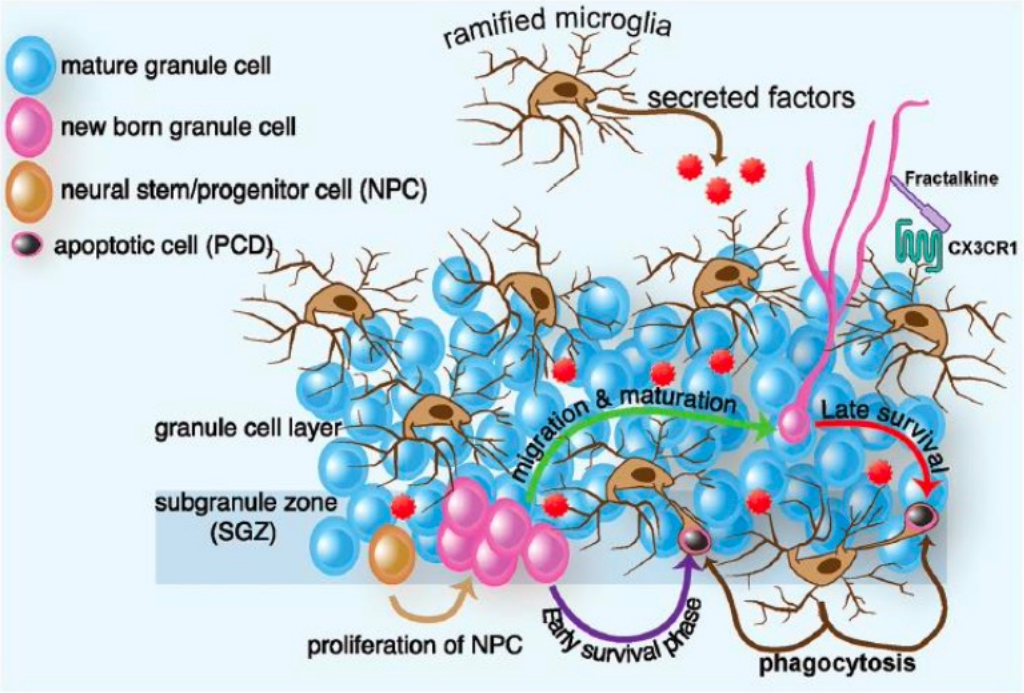
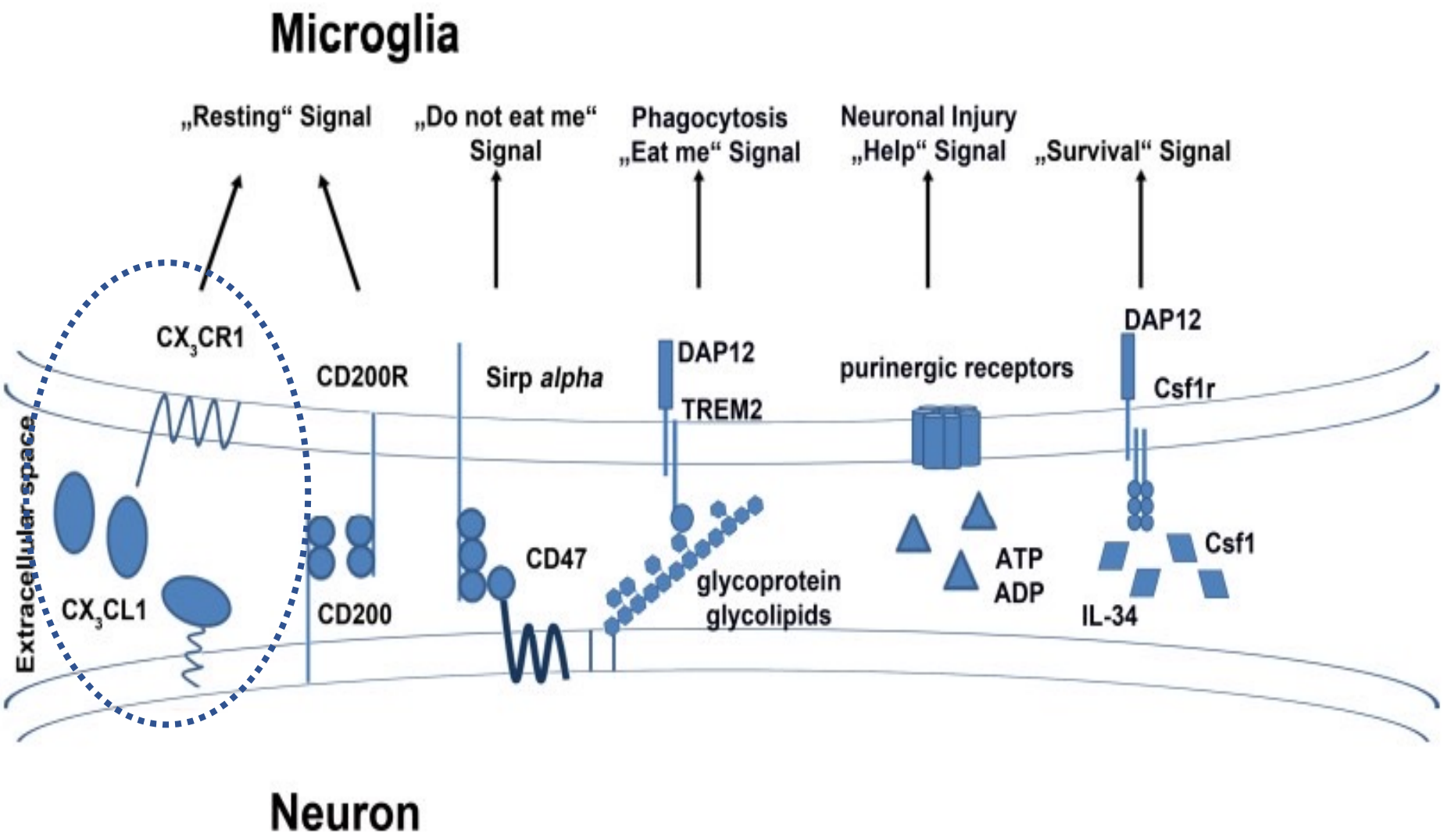


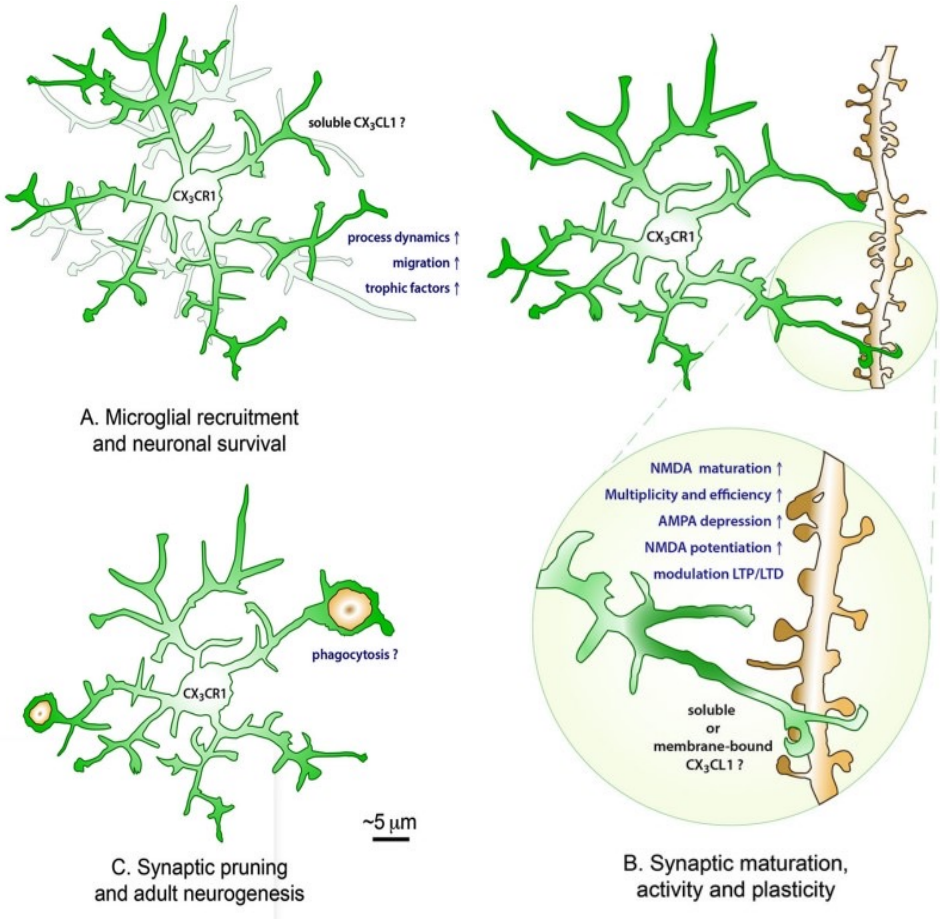
FIGURE 1 | Schematic diagram of ramified microglia and their effect on adult hippocampal neurogenesis. In intact brain, microglia regulate several steps of adult hippocampal neurogenesis. In the SGZ, progenitor cells migrate to the granule cell layer and differentiate into a neuronal phenotype, with 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
doi: 10.3389/fncel.2013.00229

Microglia – neuron crosstalk



Microglia – neuron crosstalk



Paolicelli et al., 2014

Synaptic Pruning by Microglia Is Necessary for Normal Brain Development

Rosa C. Paolicelli,[1] Giulia Bolasco,[1] Francesca Pagani,[2] Laura Maggi,[2] Maria Scianni,[2] Patrizia Panzanelli,[3] Maurizio Giustetto,[3,4] Tiago Alves Ferreira,[1] Eva Guiducci,[1] Laura Dumas,[1] Davide Ragozzino,[2] Cornelius T. Gross[1]*

Microglia shape presynaptic properties at developing glutamatergic synapses

Bernadette Basilico[1] | Francesca Pagani[2] | Alfonso Grimaldi[2] | Barbara Cortese[3] | Silvia Di Angelantonio[1,2] | Laetitia Weinhard[4] | Cornelius Gross[4] | Cristina Limatola[5,6] Laura Maggi[1] | Davide Ragozzino[1,6]

Microglia control glutamatergic synapses in the adult mouse hippocampus

Bernadette Basilico[1] | Laura Ferrucci[1] | Patrizia Ratano[2] | Maria T. Golia[1] | Alfonso Grimaldi[3] | Maria Rosito[3] | Valentina Ferretti[4] | Ingrid Reverte[1,5] | Caterina Sanchini[1,3] | Maria C. Marrone[6] | Maria Giubettini[3,7] | Valeria De Turris[3] | Debora Salerno[3] | Stefano Garofalo[1] | Marie-Kim St-Pierre[8,9] | Micael Carrier[8,9] | Massimiliano Renzi[1] | Francesca Pagani[3] | Brijesh Modi[6] | Marcello Raspa[10] | Ferdinando Scavizzi[10] | Cornelius T. Gross[11] | Silvia Marinelli[6] | Marie-Ève Tremblay[8,9,12] | Daniele Caprioli[1,5] | Laura Maggi[1] | Cristina Limatola[2,13] | Silvia Di Angelantonio[1,3] | Davide Ragozzino[1,5]

Microglia – neuron crosstalk

Microglial cells can sense neuronal activity

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

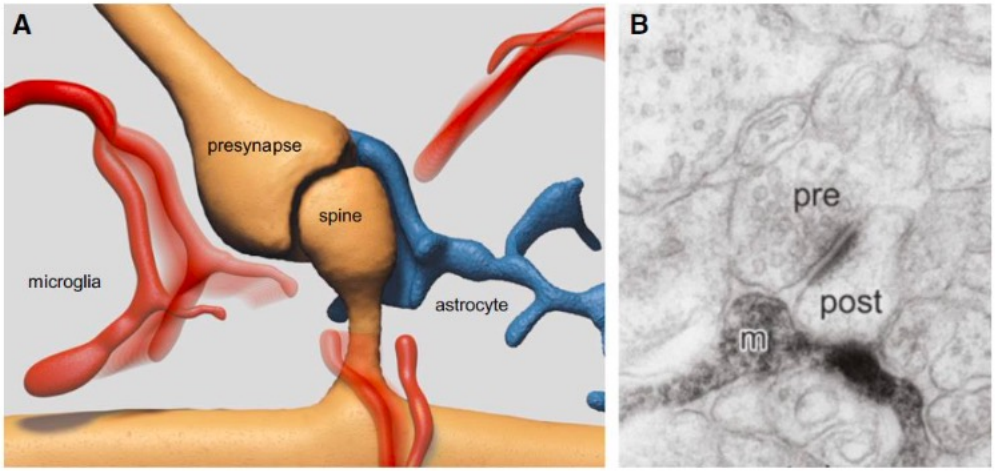


Figure 2. Dynamic Interaction of Microglial Processes with the Tripartite Synapse

(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).

Helmut Kettenmann
Neuron 77, January 9, 2013

Microglia – neuron crosstalk

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

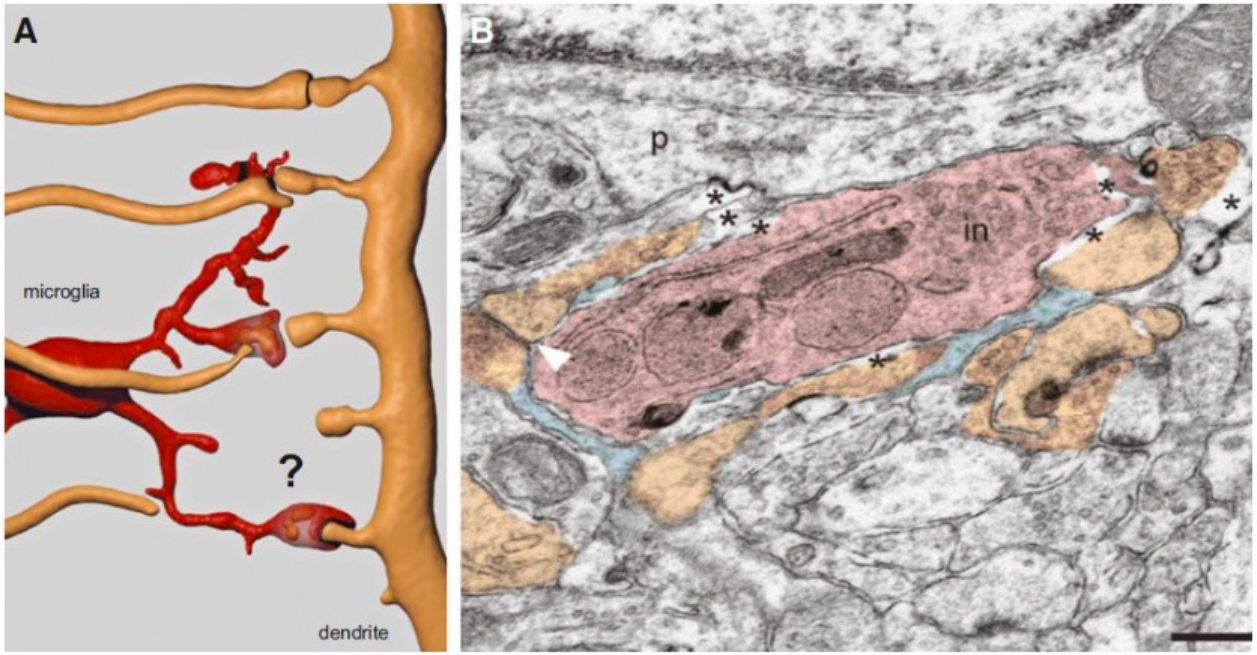


Figure 3. Synaptic Pruning by Microglial Processes

(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.

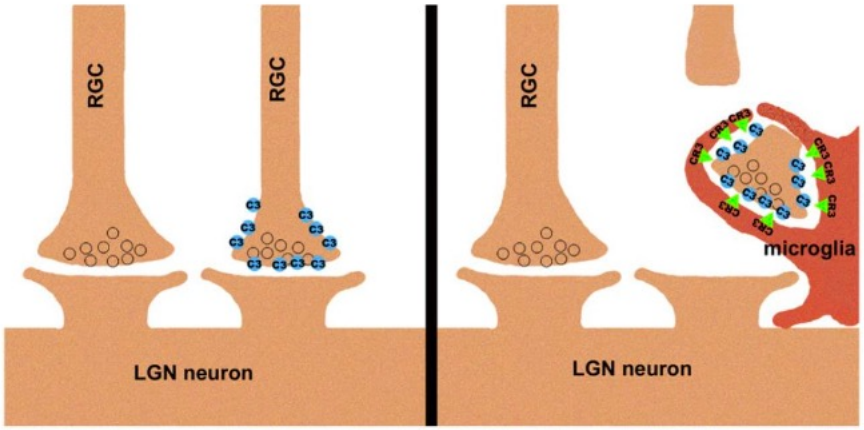
(B) The electron microphotograph shows ultrastructural interactions between microglia (red) and synapses (brown) in the mouse visual cortex. In the thickened microglial process inclusions (in) can be recognized (modified from Tremblay et al. [2010]). The asterisks indicate extended extracellular space 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.

Helmut Kettenmann Neuron 77, January 9, 2013

Microglia – neuron crosstalk

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

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



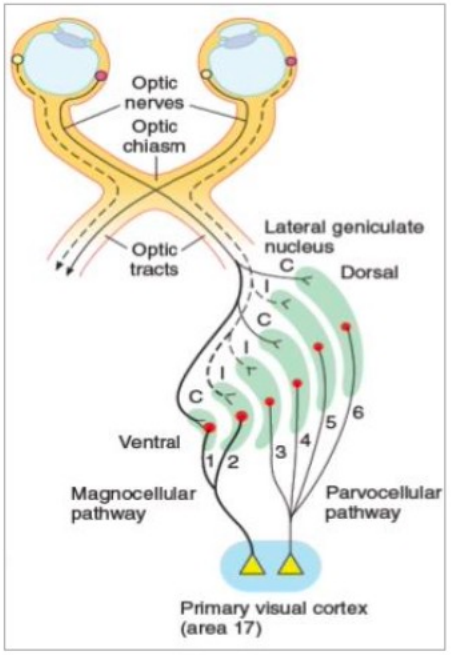
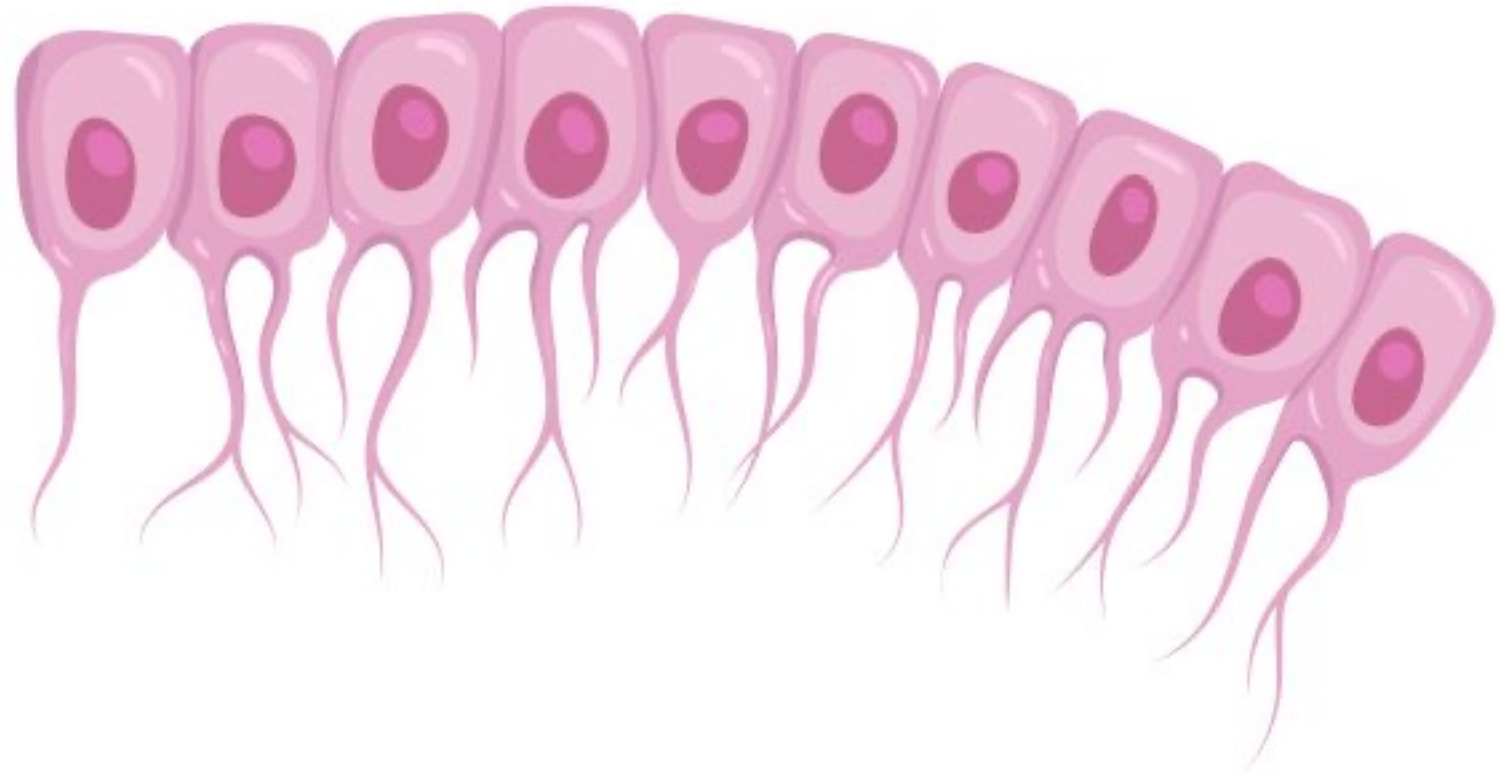


Figure 1. Microglia Phagocytose RGC Axon Material in a C3- and CR3-Dependent Manner

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**.

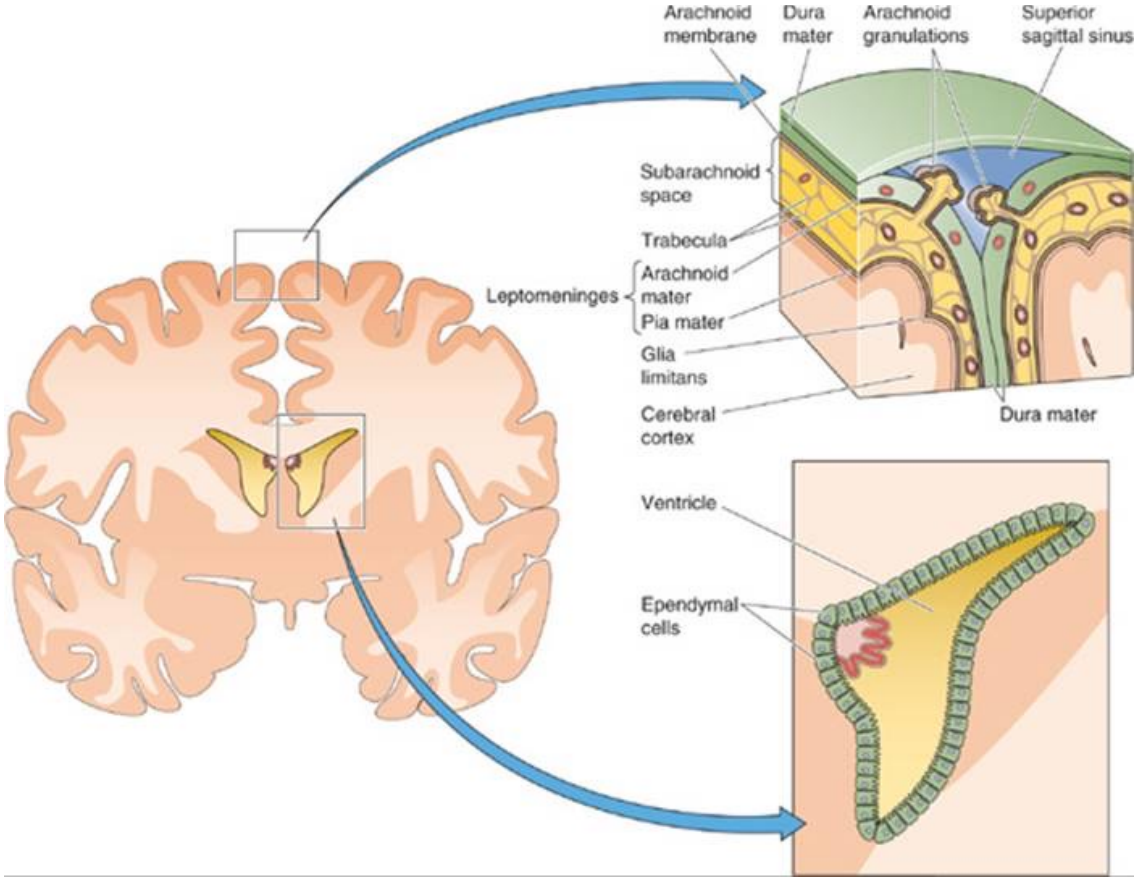
VIDEO EXPERIMENT
An Engulfment Assay: A Protocol to Assess Interactions Between CNS Phagocytes and Neurons
Dorothy P. Schafer[1], Emily K. Lehrman[1], Christopher T. Heller[1], Beth Stevens[1]
J. Vis. Exp. (88), e51482, doi:10.3791/51482 (2014)
http://www.jove.com/video/51482/an-engulfment-assay-protocol-to-assess-interactions-between-cns

Types of glial cells: ependymal cells



Types of glial cells: ependymal cells

- Line the cavities of the CNS and make up the walls of the ventricles
- Create and secrete cerebrospinal fluid(CSF)
- Beat their cilia to help circulate that CSF
- Make up the Blood-CSF barrier.
- Can act as neural stem cells



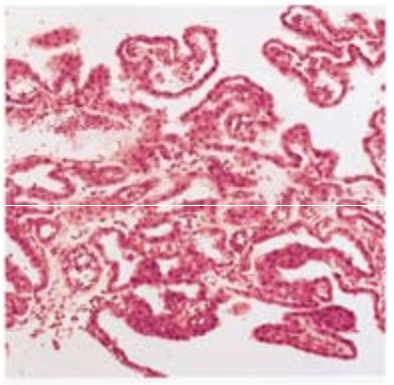
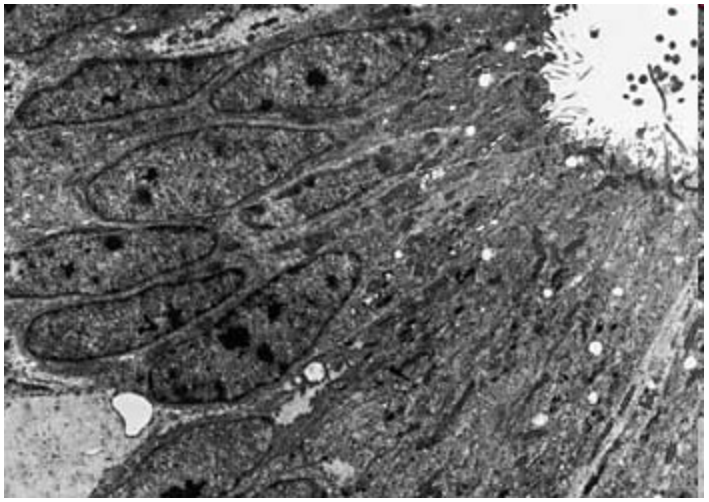


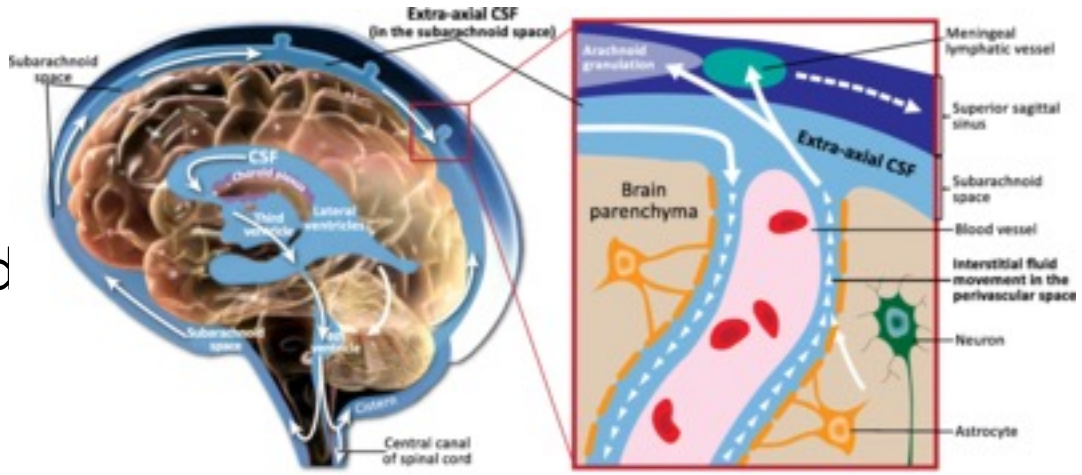
Figura 9-28

Types of glial cells: ependymal cells

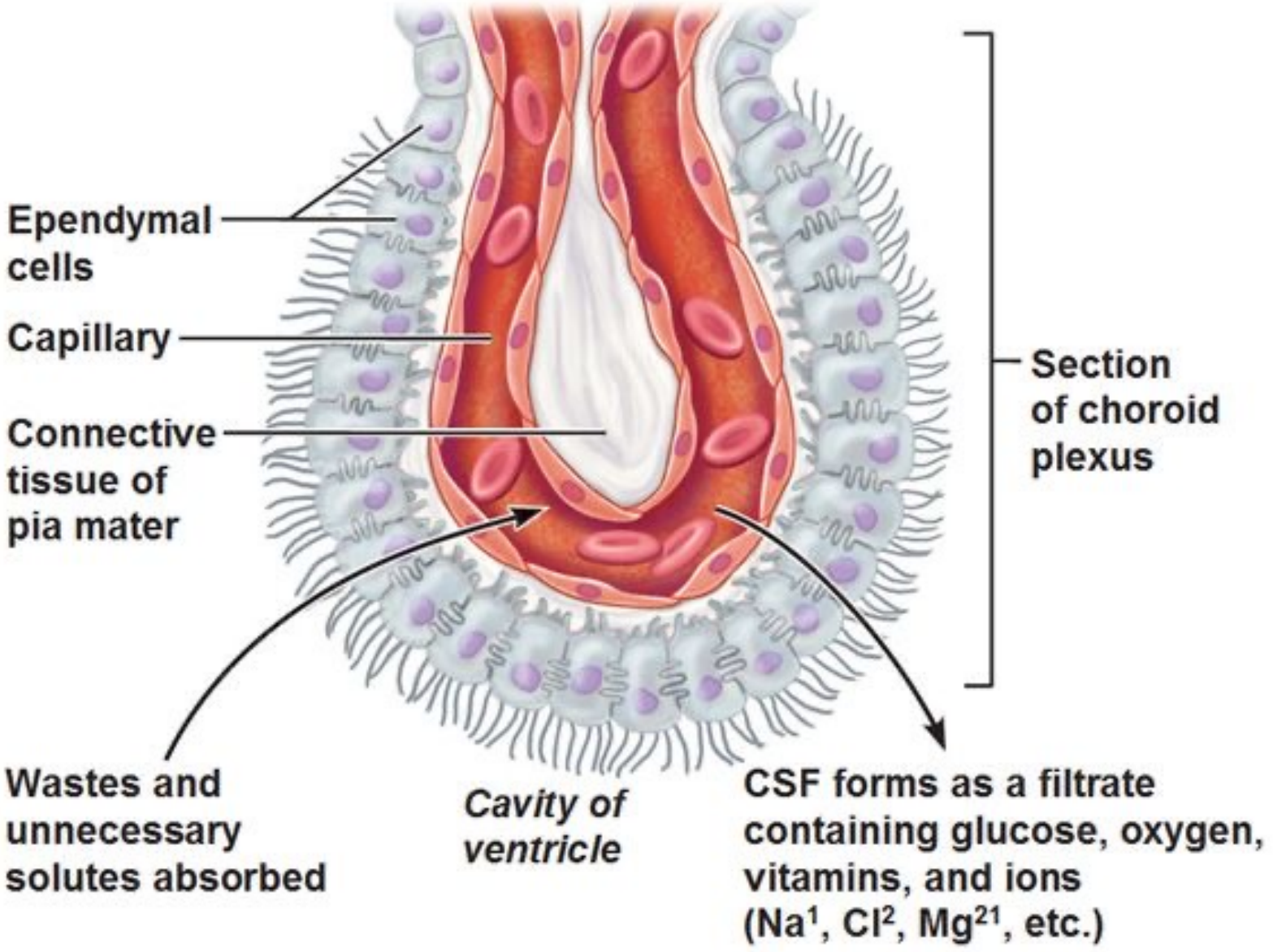
Ependymal cells
Line the ventricular system
Apical cillia circulate CSF
Apical microvilli absorb CSF



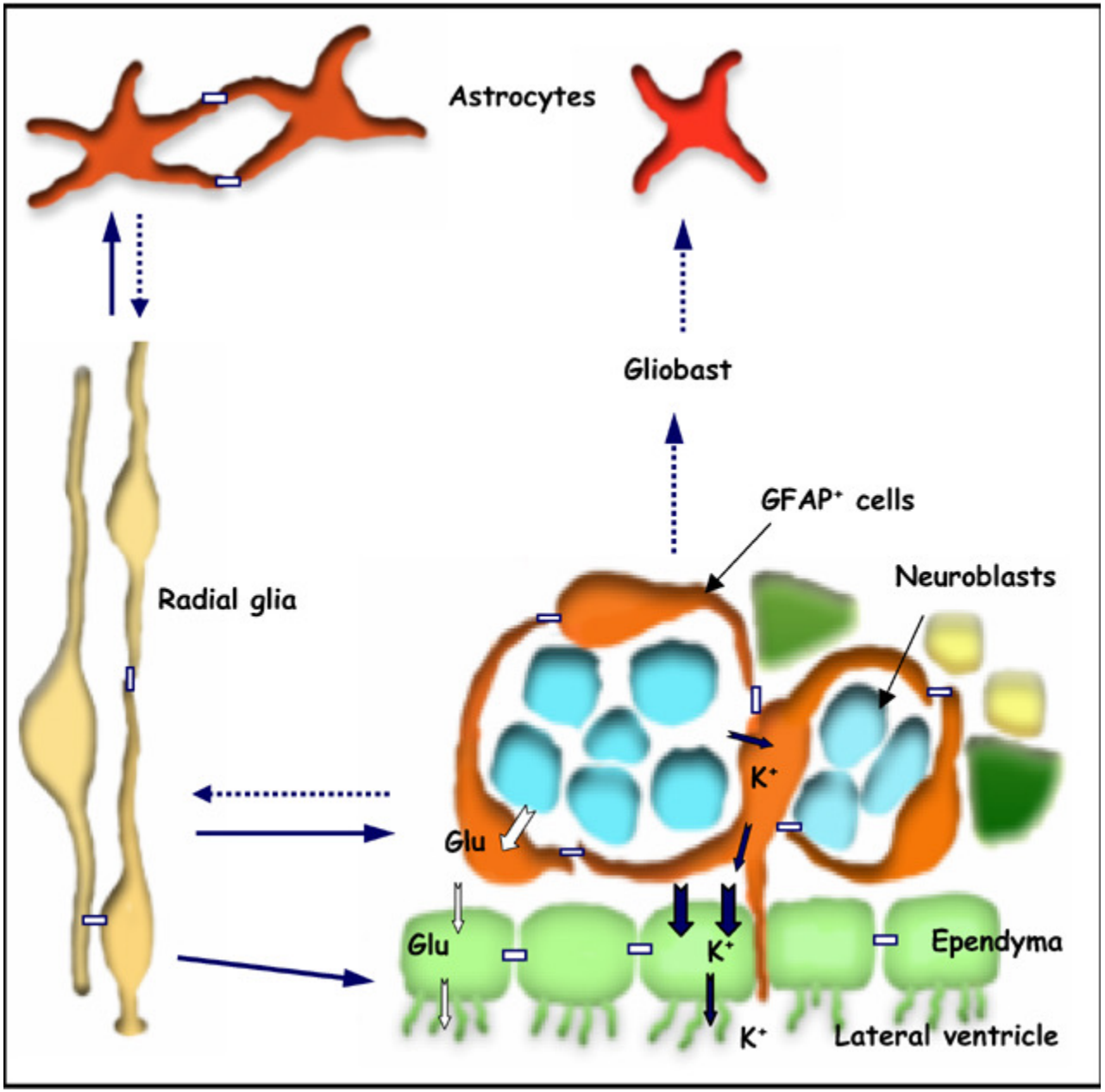
Choroidal cells
Choroid plexus abuts the ventricular system
Modified ependymal cells and capillaries
Secrete cerebrospinal fluid



Cerebrospinal fluid (CSF) – Choroid plexus



Ependymal cells



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