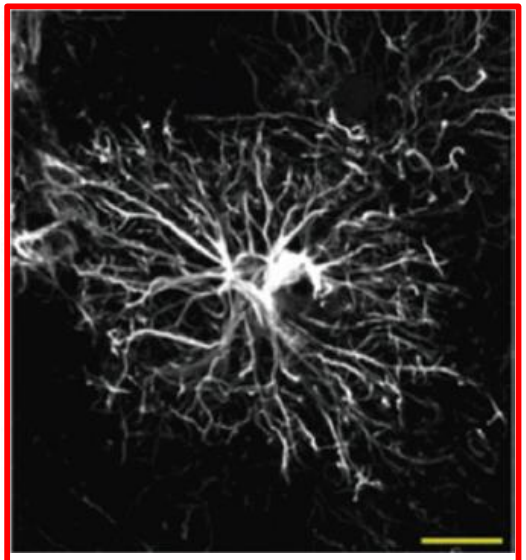
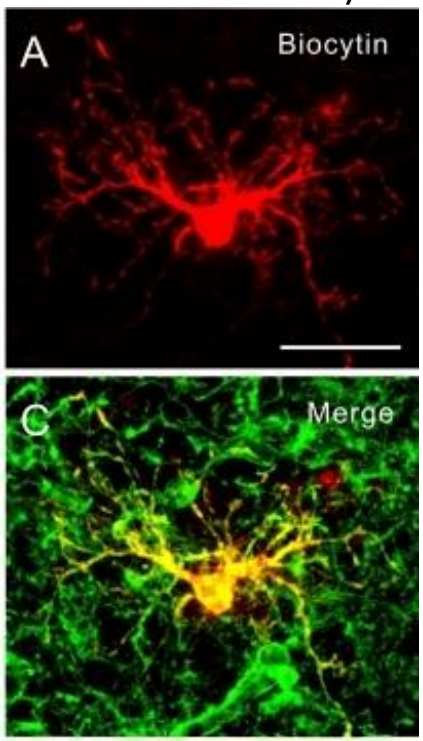
Non-myelinating glia in the CNS: ASTROCYTES, MICROGLIA, OPCs

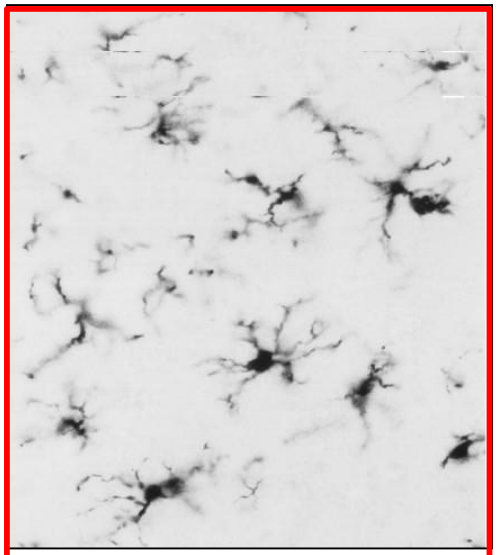


astrocyte

OPCs (Oligodendrocyte Precursor Cells)



Green: NG2 immunostaining

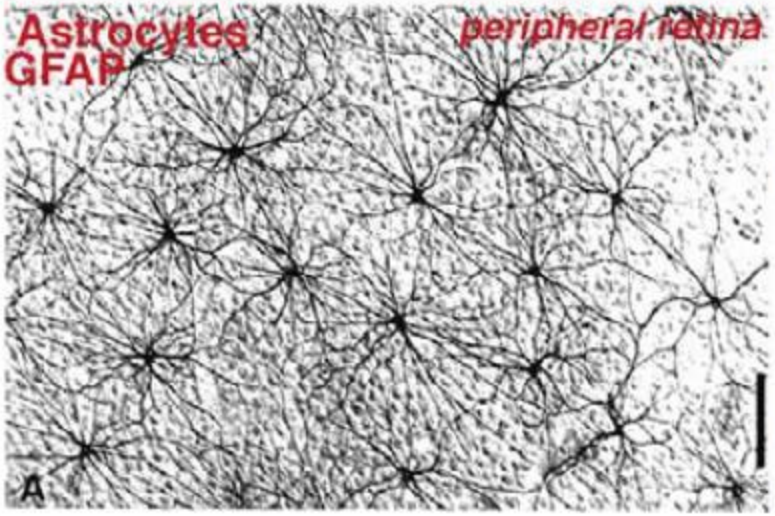


microglia

"What is the function of glial cells in neural centers? The answer is still not known, and the problem is even more serious because it may remain unsolved for many years to come until physiologists find direct methods to attack it"
Santiago Ramon-y Cajal (1909/1911)

Astrocytes (astroglia) "star-cells"

Most numerous cell type in brain
Constitute ~30-50% of brain volume



NORMAL FUNCTIONS

Homeostasis of neuronal microenvironment
- Ionic
- Metabolic
- Neurotransmitter uptake

Blood-Brain barrier: induction and maintenance

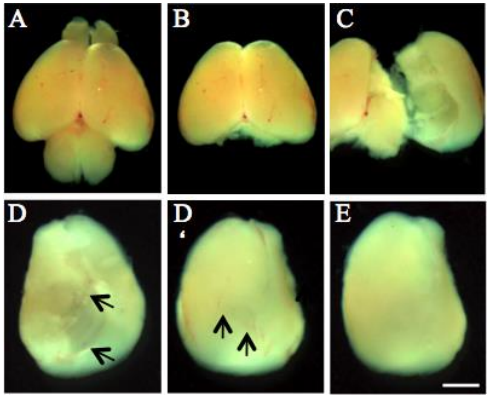
Trophic support of neurons (growth factors)

Synaptogenesis and synaptic remodeling

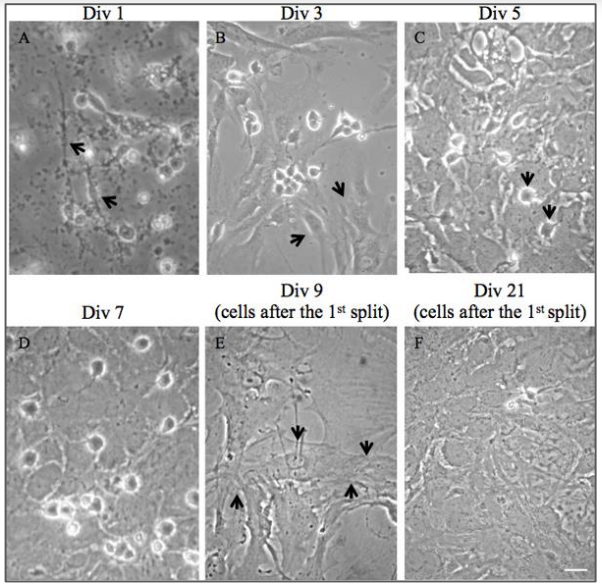
New role! → Gliotransmission: synaptic modulation

VIDEO: Isolation and Culture of Mouse Cortical Astrocytes

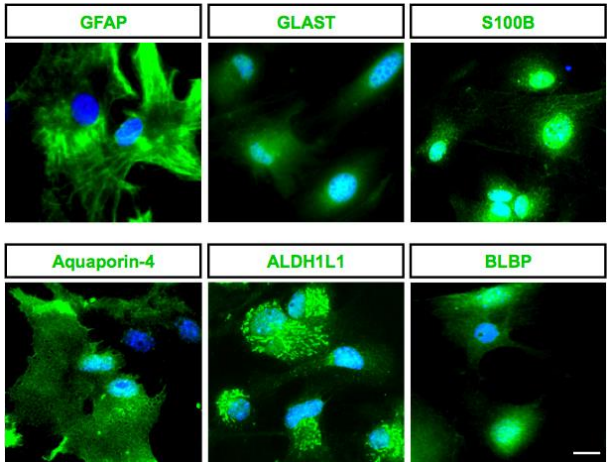
http://www.jove.com/video/50079/isolation-and-culture-of-mouse-cortical-astrocytes



Dissection of postnatal (P3) mouse cortex. A) Whole brain. B) Brain after removal of olfactory bulbs and cerebellum. C) Isolation of cortices by peeling off the plate-like structure of the cortex from the brain. D, D') Cortex from ventral and dorsal site with meninges (black arrows indicate meningeal arteries). E) Cortex without meninges. Scale bar, 1.5 mm.



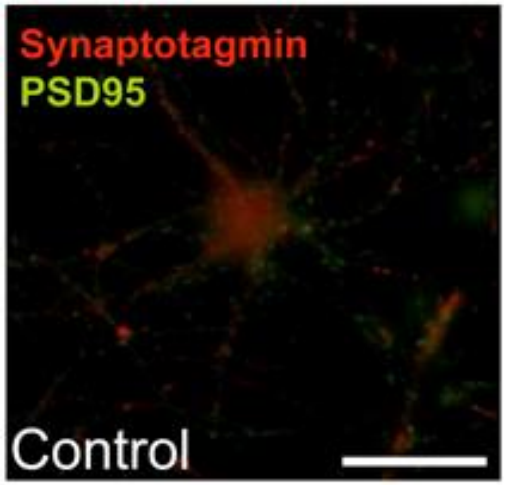
Morphological overview of isolated mixed cortical cells and pure astrocyte culture at different timepoints after isolation. A) 1 day after plating of mixed cortical cells. First astrocytes are attached to the bottom of the flask (black arrows) and dying neurons are in the supernatant. B) 3 days after plating of mixed cortical cells. Astrocyte layer is forming (black arrows). Neurons are almost absent. C) 5 days after plating of mixed cortical cells. First microglia and OPCs on top of a astrocyte layer (black arrows). D) 7 days after plating of mixed cortical cells. Astrocyte layer is completely confluent. E) After removing microglia and OPCs by vigorous shaking and 2 days after splitting, attached cells show astrocyte morphology with low density (arrows indicate one cell). F) Astrocyte layer shows high density 2 weeks after the first split. Scale bar, 10 µm.



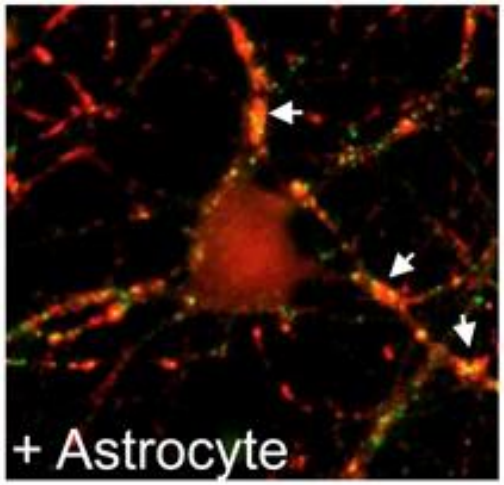
Purity of primary astrocyte culture. Immunolabeling of primary mouse astrocyte cultures with the markers GFAP, GLAST, S100B, Aquaporin-4, ALDH1L1 and BLBP (all green) revealed pure primary astrocyte culture. Nuclei are stained with 4',6'-diamidino-2-phenylindole (DAPI) (blue). Scale bar: 10 µm.

Schildge et al., 2013, J. Vis. Exp. doi:10.3791/50079 (2013)

Astrocytes play active roles in the formation of synapses



Purified retinal ganglion cells (RGCs) survive in culture, but show little spontaneous synapse activity and form few synapses



RGS cultured in the presence of a feeding layer of astrocytes or astrocyte-conditioned medium show ~10-fold more excitatory synapse activity and 5-7-fold increase in the number of synapses.

Eroglu et al., 2009, Cell
DOI 10.1016/j.cell.2009.09.025

Human astrocytes are larger…….

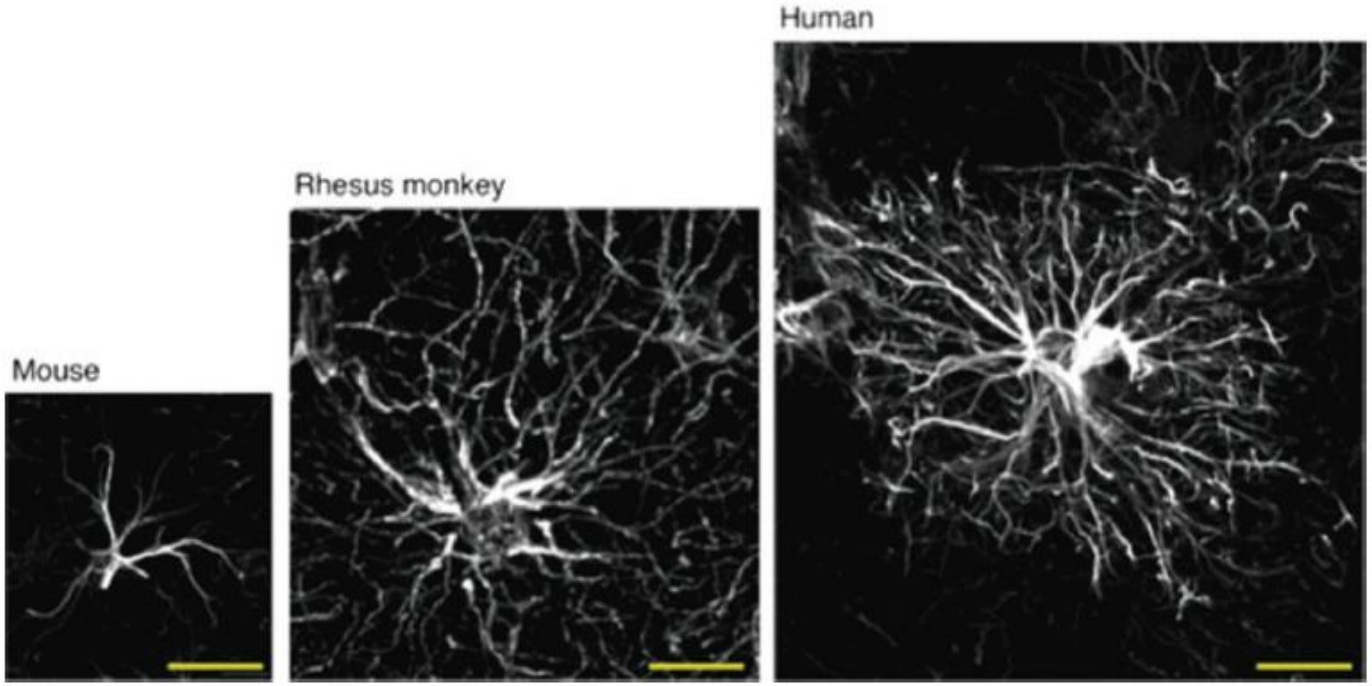
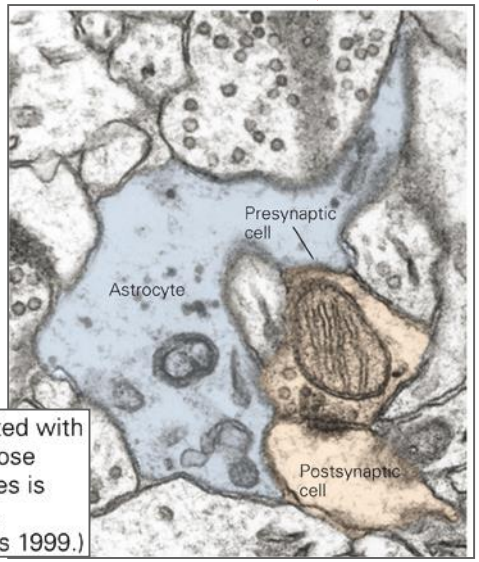


Fig. 6.
Human astrocytes are larger and more complex than rodent and other primates. Mouse, Rhesus Monkey, and Human astrocytes are compared by GFAP staining (*white*). Scale = 20 μm.

Oberheim et al., 2012
Methods Mol Biol. 814: 23–45.
doi:10.1007/978-1-61779-452-0_3

- Astrocytes contact virtually every cell component in brain
  - Other astrocytes (gap junctions)
  - Ependymal cells
  - Neurons (somas, processes, synapses)
  - Oligodendroglia
  - Capillary endothelial cells

Astrocytes wrap around synapses and are in close contact with neurons:



C. The processes of astrocytes are intimately associated with both presynaptic and postsynaptic elements. **1.** The close association between astrocyte processes and synapses is seen in this electron micrograph of hippocampal cells. (Reproduced, with permission, from Ventura and Harris 1999.)

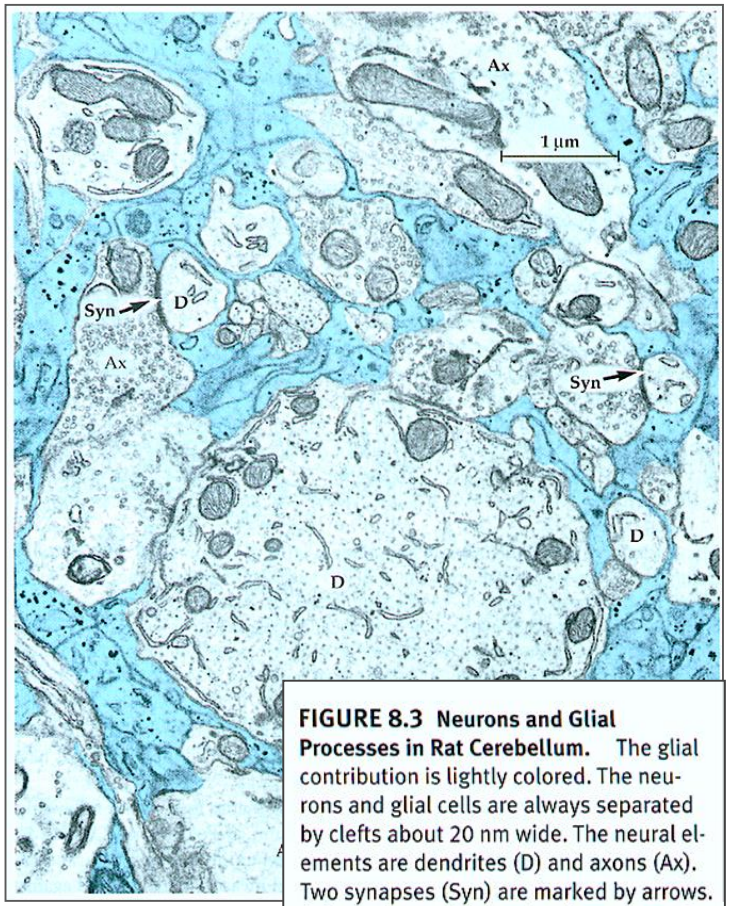
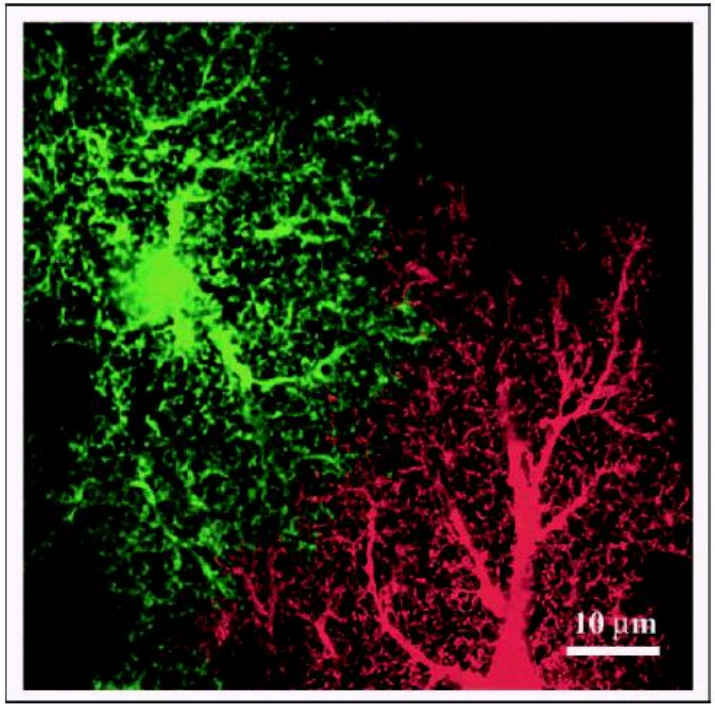
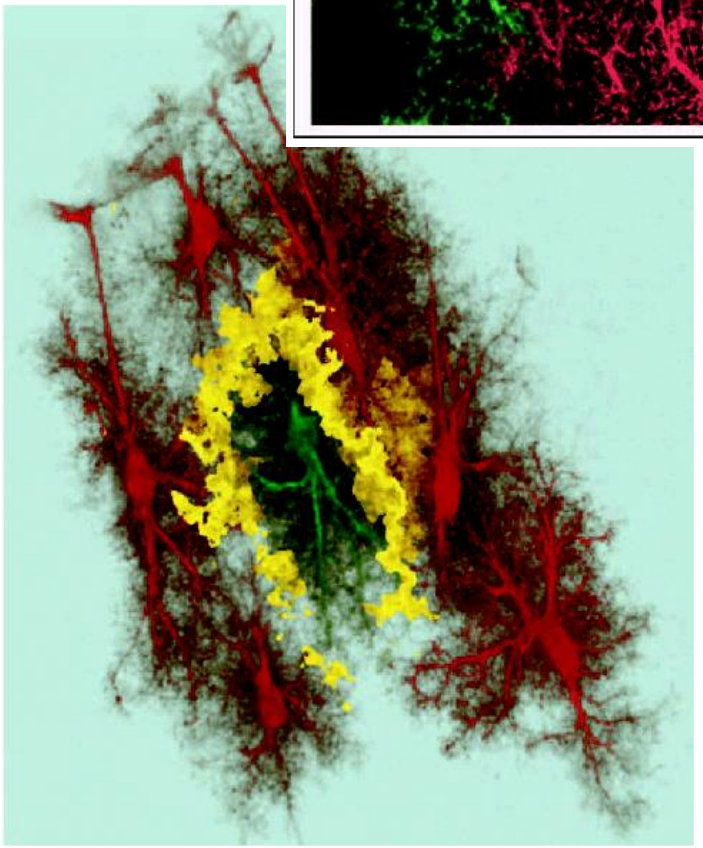


FIGURE 8.3 Neurons and Glial Processes in Rat Cerebellum. The glial contribution is lightly colored. The neurons and glial cells are always separated by clefts about 20 nm wide. The neural elements are dendrites (D) and axons (Ax). Two synapses (Syn) are marked by arrows. (After Peters, Palay, and Webster, 1991.)

Individual astrocytes occupy distinct domains

The intermingling of protoplasmic astrocytes in the hippocampal CA1 molecular layer was examined by filling adjoining cells with different coloured fluorescent dyes (Alexa 468, a green fluorescent dye, and Alexa 488, a red fluorescent dye) by microinjection. The discrete region of interaction of the fine terminal processes was revealed (yellow) by first blurring the images slightly (using a Gaussian blur filter) and then remapping the colour of the resultant area of overlap to bright yellow. This shows where the fine terminal processes of the adjoining astrocytes are closest to one another, although not actually overlapping. The 'boundary' of each astrocyte has a distinct surface that abuts neighbouring astrocytes. The long thin processes that extend from each cell shown in this figure are the 'siphon' processes of the astrocytes, which end in sheet-like surfaces that line the adjacent blood vessel. Image courtesy of E. Bushong and M. Ellisman, The National Center for Microscopy and Imaging Research, University of California, San Diego, USA.





Volterra & Meldolesi, 2005
Nature Reviews Neurosci
doi:10.1038/nrn1722

Astrocyte domain organization in pathological states

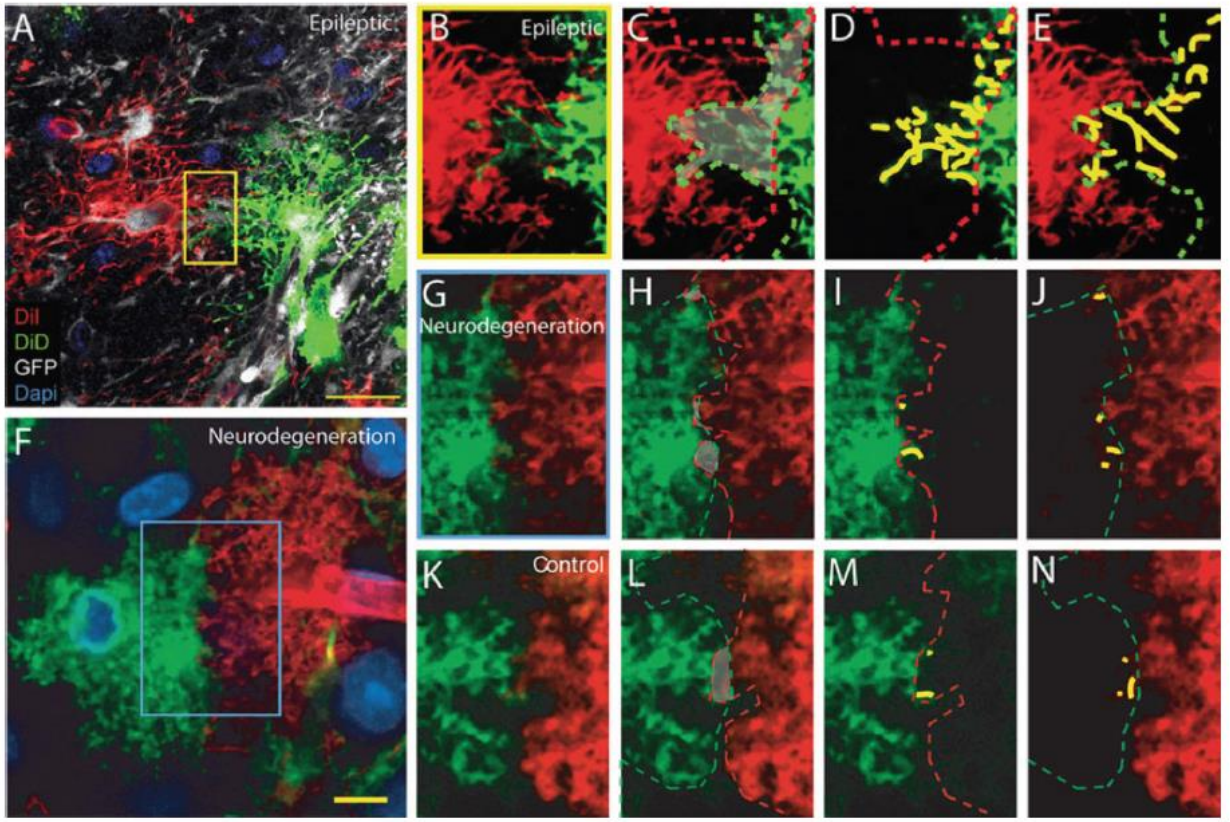
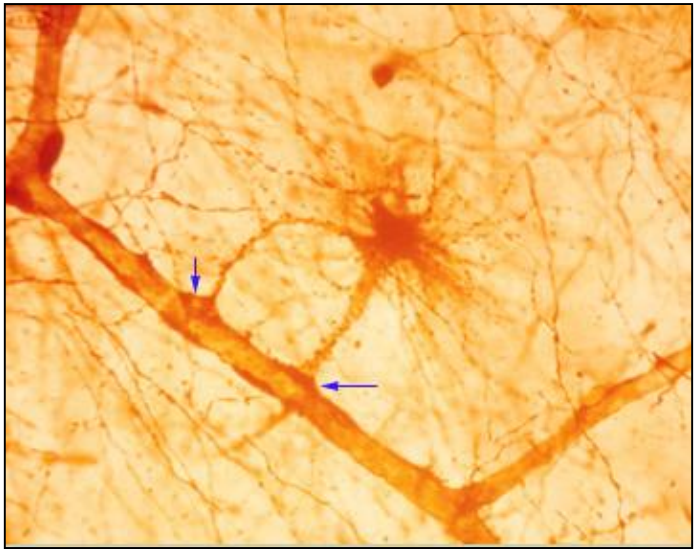


Fig. 2.
Astrocytic domain organization varies with pathology. The domain organization of protoplasmic astrocytes is lost in epileptic brains, but maintained in neurodegeneration. (**a**) Reactive astrocytes 1 week post-iron injection lose the domain organization. Diolistic labelling of the cortex of a GFAP-GFP mouse 1 week post-iron injection near injection site. Two adjacent GFP positive astrocytes are labeled with DiI and DiD. DAPI, *blue*, GFP, *green*, DiI, *red*, DiD, *white*. (**b**–**e**) High power of yellow box in (**a**). area of overlap delineated in *grey*, *red line* is border of the domain of the *red* cell, *green line* is the border of the domain of the *white* cell. (**g**–**h**) *Yellow lines* indicate the processes of the cell that pass into the domain of the adjacent cell's domain represented by the dotted line. (**f**) Cortical astrocytes in an Alzheimer disease model Tg2576 become reactive, but do not lose the domain organization. Diolistic labelling of cortical astrocytes in Tg2576 mouse. (**g**–**j**) High power of *blue box* in (**f**) showing limited overlap between adjacent cells. (**k**–**n**) Adjacent control astrocytes demonstrating the domain organization. Scale: (**a**) 20 μ m; (**g**–**h**) 10 μ m. From (22).

Oberheim et al., 2012
Methods Mol Biol. 814: 23–45.
doi:10.1007/978-1-61779-452-0_3

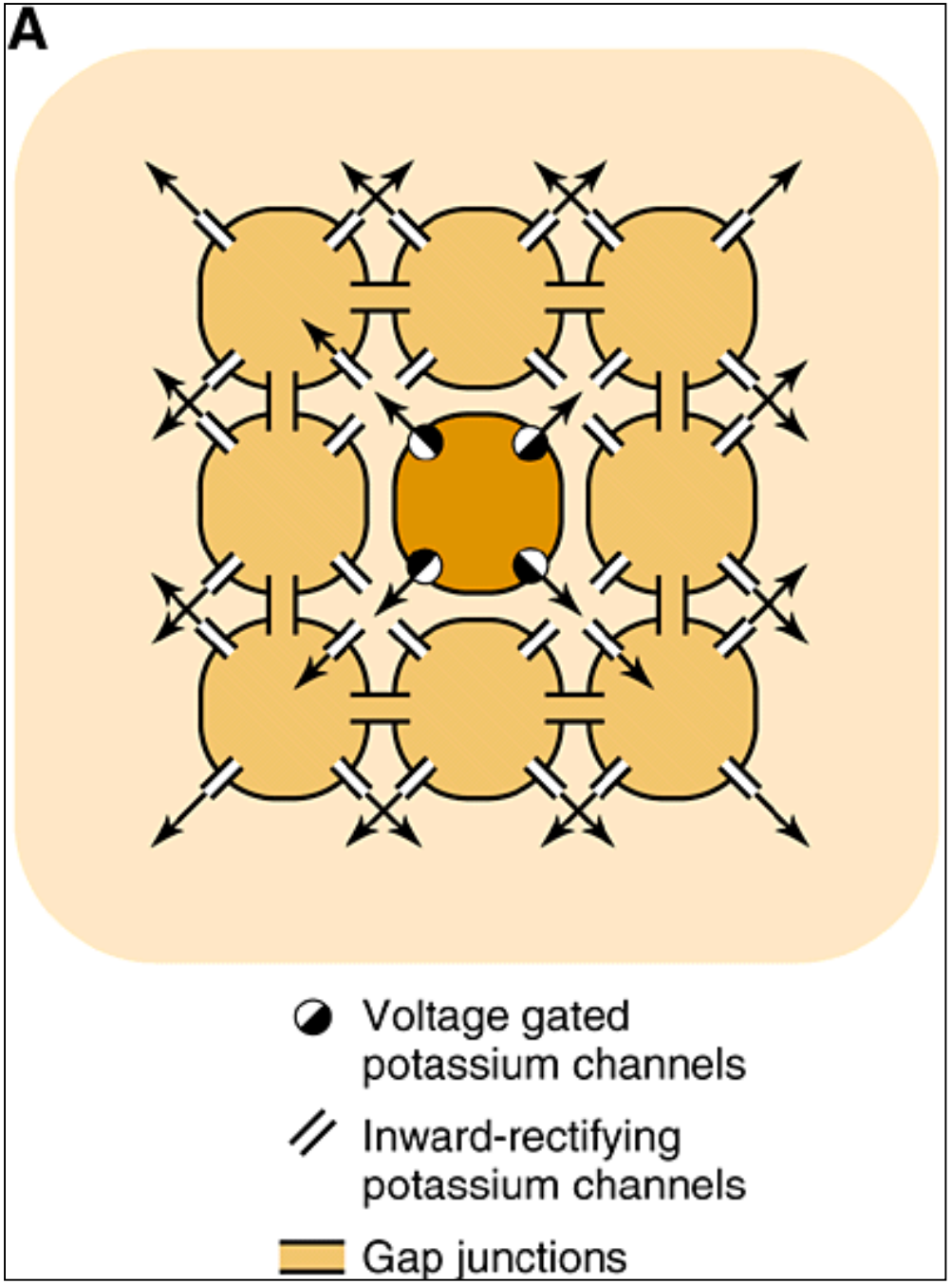
Astrocytes and brain homeostasis– regulation of blood flow



- Numerous fine processes of astrocytes form close associations with capillaries and neurons.
- Enhanced neuronal activity causes astrocytes to signal to blood vessels for regional increases in blood flow.
- Results in enhanced delivery of oxygen and glucose to the active brain regions.

Spatial buffering by astrocytes

This conceptual diagram indicates the pathways available for potassium ions to diffuse through the glial syncytium (light orange) subsequent to their release from neuronal membranes (dark orange) during neural acivity.



Elimination of Glu and GABA from the intersynaptic space by astrocytes

Glu = glutamate

GS = glutamine sintethetase

Gln = glutamine

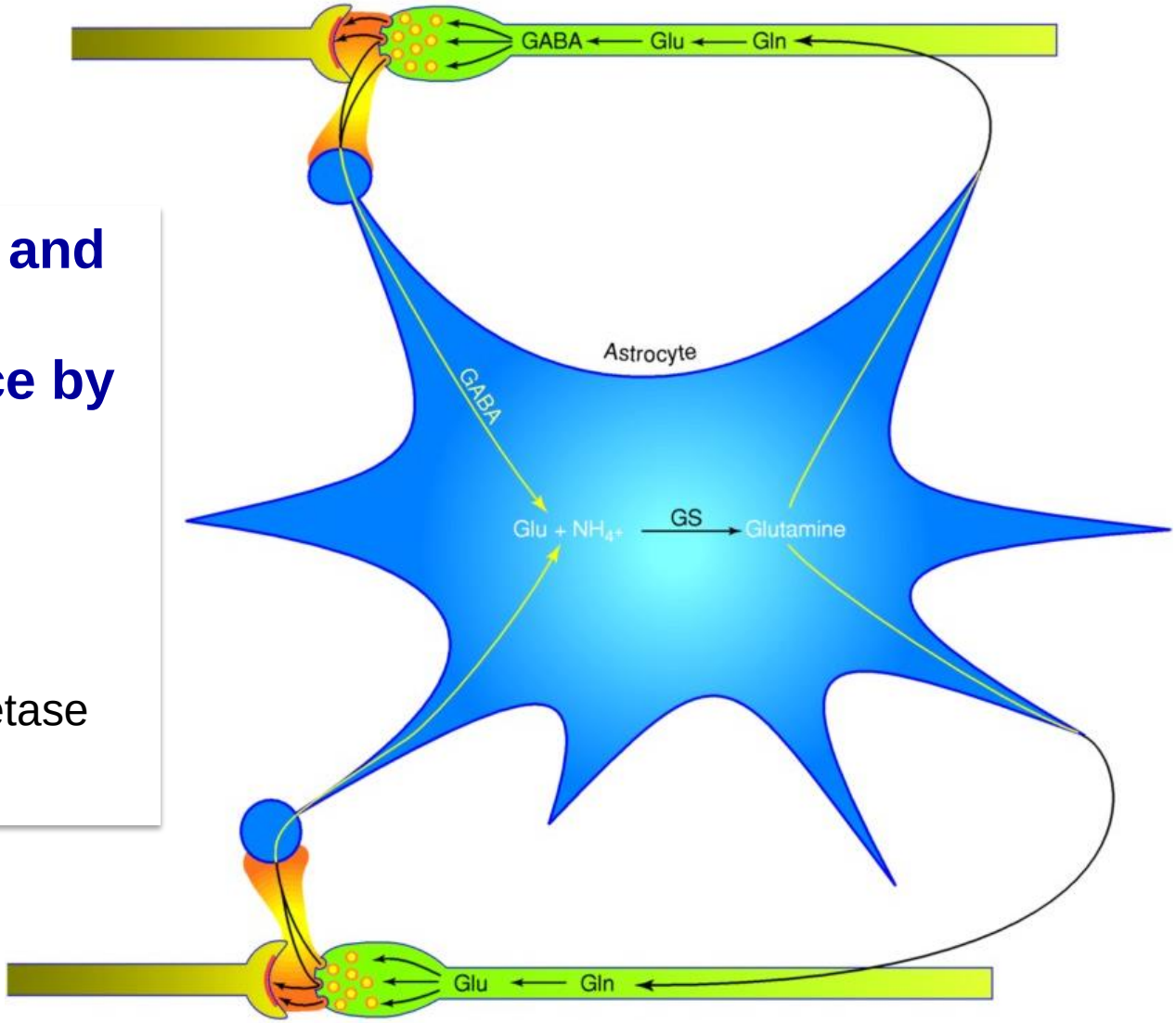


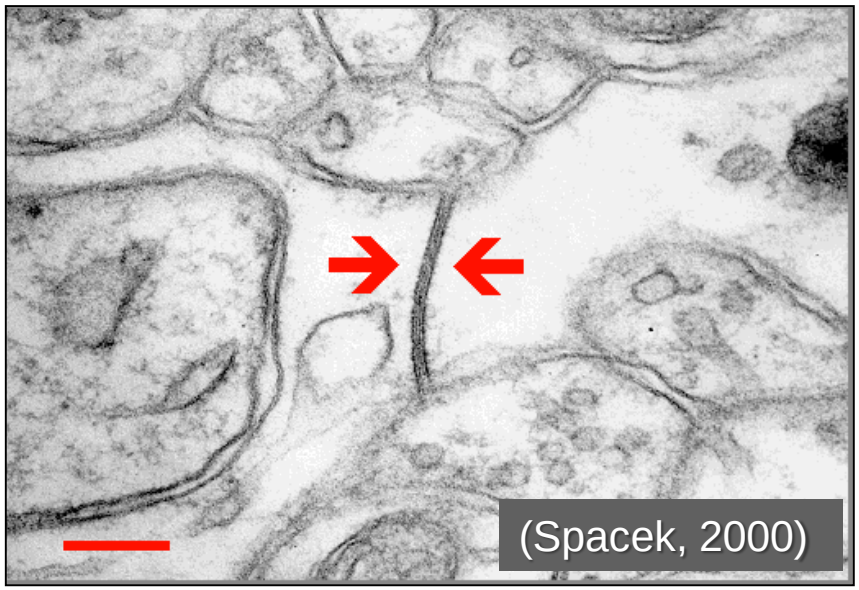
FIGURE 19 The glutamate–glutamine cycle is an example of a complex mechanism that involves an active coupling of neurotransmitter metabolism between neurons and astrocytes. The systems of exchange of glutamine, glutamate, GABA, and ammonia between neurons and astrocytes are highly integrated. The postulated detoxification of ammonia and the inactivation of glutamate and GABA by astrocytes are consistent with the exclusive localization of glutamine synthetase in the astroglial compartment.

The new concept of "GLIOTRANSMISSION"

Astrocytes are now viewed as 'excitable' cells in the sense that, when activated by internal or external signals, they deliver specific messages to neighbouring cells — an activity that has been called 'gliotransmission'. However, astrocytes cannot generate action potentials. Their excitation, which is chemically encoded, can be revealed not by electrophysiology, as in neurons, but by assays of $[Ca^{2+}]i$ transients and oscillations.

Two main forms of astrocyte excitation are well documented: one that is generated by chemical signals in neuronal circuits (neuron-dependent excitation) and one that occurs independently of neuronal input (spontaneous excitation).

Astrocyte-astrocyte communication



Cells are linked together by gap junctions

When glial cells are coupled by gap junctions, calcium waves can spread from cell to cell in a continuous progresson

Stimulation of one astrocyte can cause a calcium response in a subset of neighboring astrocytes, but not others, suggesting distinct networks of astrocytes.

Astrocyte-neuron communication

Glial cells “sense” neuronal activity and respond to neurotransmitter molecules released during synaptic transmission by increasing intracellular calcium release

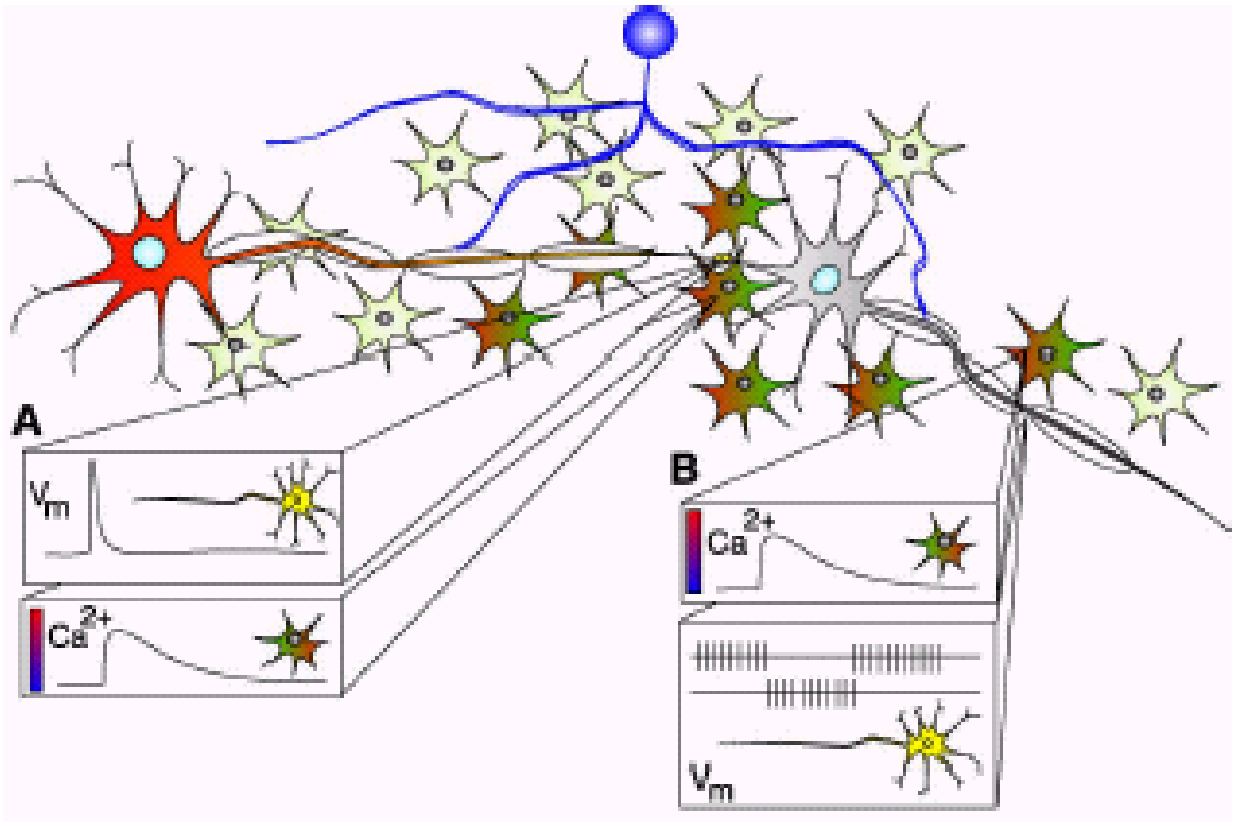
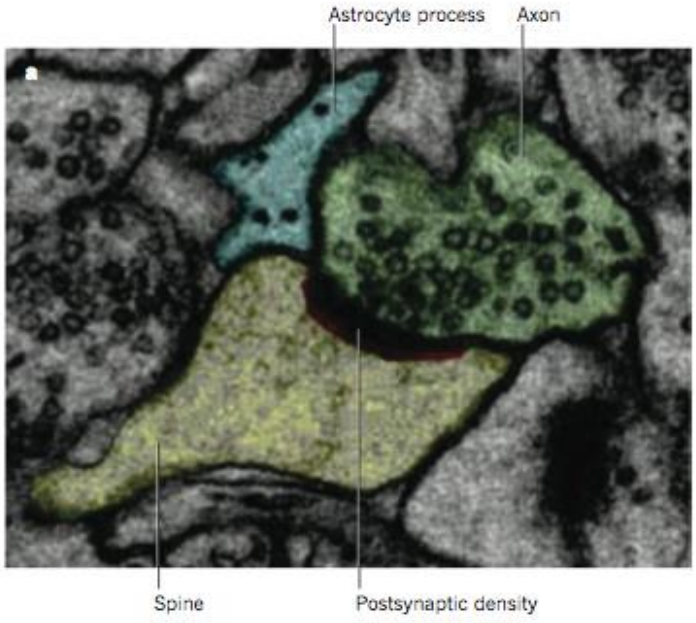
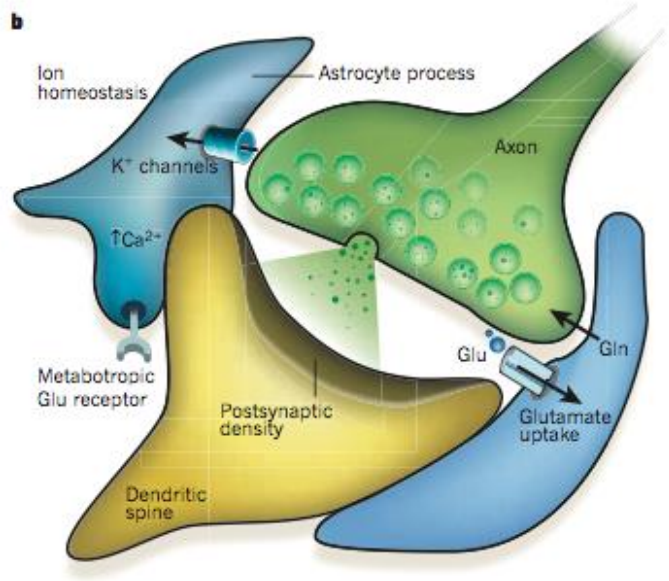


Fig. 2. Calcium imaging reveals communication between neurons and glia. (A) Molecules released during synaptic transmission bind receptors on glia that cause increases in intracellular Ca^{2+} (rainbow colored cells), which are propagated as waves through glial networks. (B) Increases or decreases in axonal firing may coincide with the passage of a glial Ca^{2+} wave. Oligodendrocytes (purple) myelinate CNS axons. v_m, membrane voltage.

Fields & Stevens-Graham, 2002
Science, 298:556-562





Eroglu and Barres, 2010, Nature doi:10.1038/nature09612)

The tripartite synapse

- Individual astrocytes can make contact with and ensheath 100s-1000s of synapses.
- Astrocytes possess many of the same neurotransmitter receptors as neurons.
- Neurotransmitter release by neurons activates calcium-based signaling cascades in astrocytes.
- Astrocytes then release neuroactive substances back to neurons to be used to make more neurotransmitters.
- Also maintain appropriate ion concentration of extracellular fluid surrounding neurons by taking up excess potassium

"GLIOTRANSMITTERS"

Table 1

Emerging substances released by astrocytes

Substance	Mechanism(s) of release	Function	Ref.
Neurotransmitters			
Glutamate	Exocytosis[a,c] Plasma membrane channels: connexin (Cx) hemichannels Transporters: P2X7[a,c]; glutamate-cysteine antiporter[a,c] and excitatory amino acid transporters1/2 (EAAT1/2)[a]	Modulation of glutamate ionotropic and metabotropic receptors on neurons and glia[a,c]	[22,47–55,56••]
GABA	Plasma membrane channels: Best1 anion channel[a,c] Transporters: gamma-aminobutyric acid (GABA) GAT1 (SLC6A1) and GAT3 (SLC6A11) transporters[a,c]	Modulation of $GABA_A$ and $GABA_B$ receptors on neurons and glia[a,b]	[57,58]
Adenosine/ATP	Exocytosis[a,b] Plasma membrane channels: Cx or pannexin (Panx) hemichannels Transporters: P2X7 receptors ($P2X_7Rs$) and other anion channels[a,c,b]	Modulation of basal synaptic transmission by presynaptic A_{2A} receptor. It also has excitatory (P2X receptor) and pleiotropic effects (P_2Y) on neuron and glia cells[a,b]	[59–62]
Glycine	Transporters: glycine transporter GlyT1 (SLC6A9)	Inhibitory effects on neurons[a,b]	[69]
Neuropeptide Y	Exocytosis[a,c]	An important mediator of synaptic development and function	[32]
Neuromodulators			
D-Serine	Exocytosis[a,c] Plasma membrane channels: Panx hemichannels[a] and volume-regulated anion channels (VRCAs) Transporters: P2X7[a] and Na^+-independent alanine–serine–cysteine transporter-2 (ASCT2)[a]	Co-agonist of N-methyl-D-aspartate (NMDA) receptors. The release of D-serine from astrocytes is an important component of long term potentiation (LTP) in hippocampal Schaffer collateral-pyramidal neurons[a,c]	[63–68,96–98]

[a] In cultured cells.
[b] *In vivo*.
[c] In acute slices.

Petrelli & Bezzi 2016
Current Opinion in Pharmacology
http://dx.doi.org/10.1016/j.coph.2015.11.010

Astrocytic processes may contain synaptyc-like microvescicles

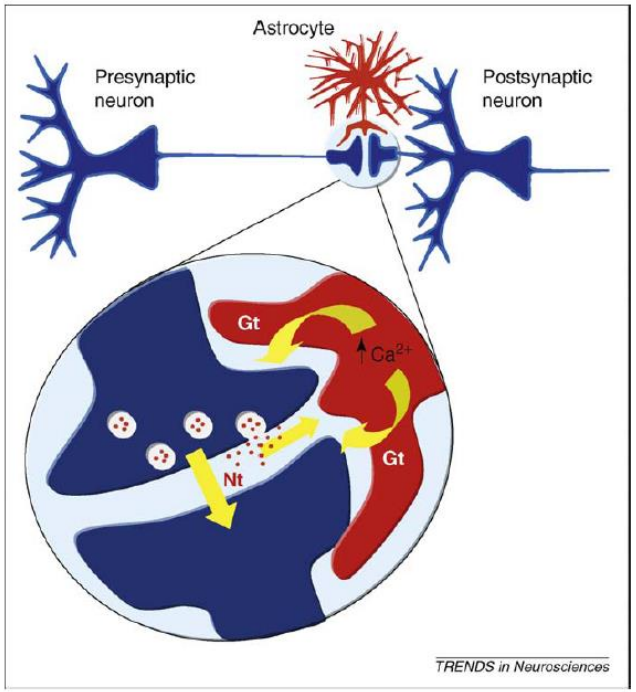
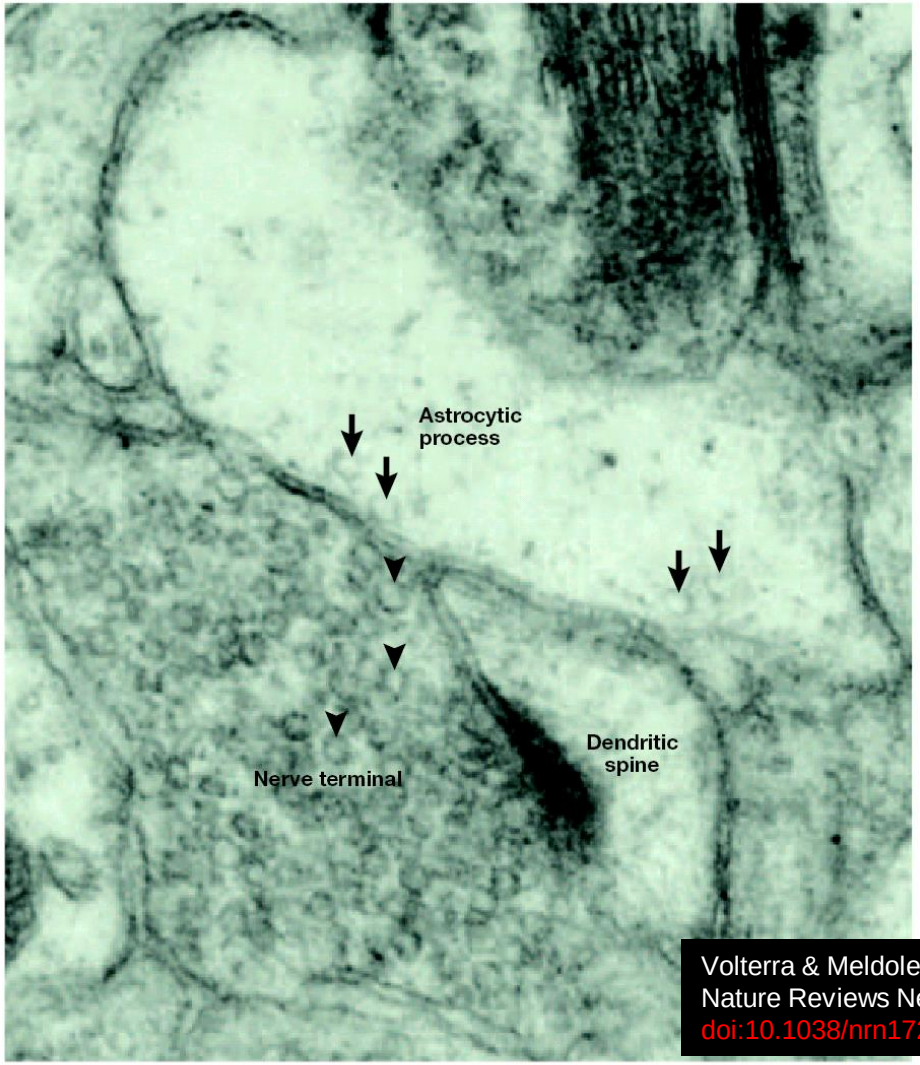


Figure 2. Scheme of the tripartite synapse. Cartoon representing the transfer of information between neuronal elements and astrocyte at the tripartite synapse. Astrocytes respond with Ca^{2+} elevations to neurotransmitters (Nt) released during synaptic activity and, in turn, control neuronal excitability and synaptic transmission through the Ca^{2+}-dependent release of gliotransmitters (Gt).

Perea et al., 2009, Trend in Neurosci.
doi:10.1016/j.tins.2009.05.001



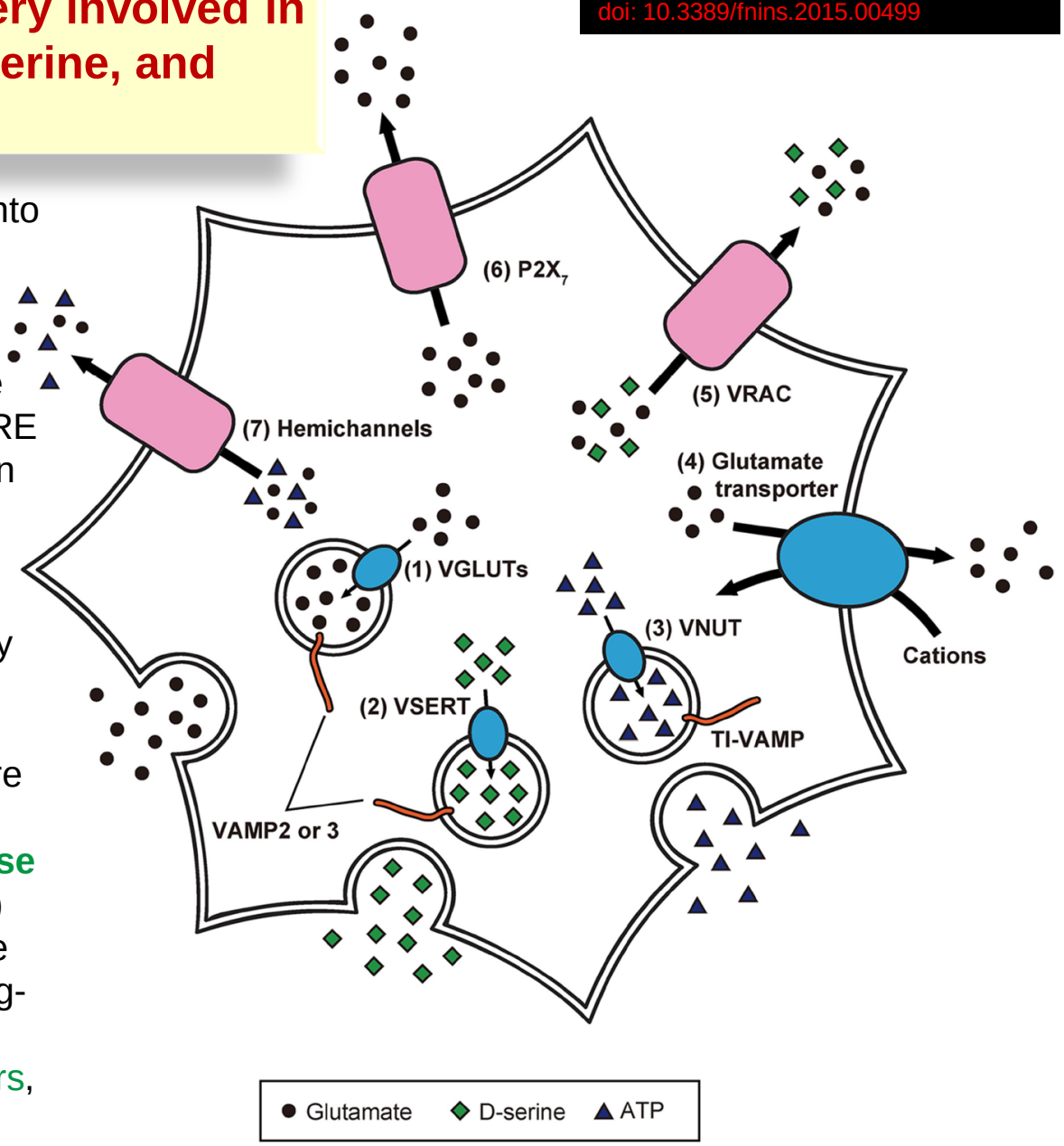
Volterra & Meldolesi, 2005
Nature Reviews Neurosci
doi:10.1038/nrn1722

Figure 3 | **Synaptic-like microvesicles in an astrocyte process facing an excitatory synapse in the hippocampus.** Electron micrograph showing synaptic-like microvesicles (SLMVs) in an astrocytic process in the outer two-thirds of the hippocampal dentate molecular layer. Arrows indicate astrocytic SLMVs. These vesicles resemble synaptic vesicles (arrowheads) in both shape and size, and are observed in close proximity to the asymmetric synaptic specialization, at extrasynaptic sites that face either the nerve terminal or a dendritic spine. To obtain better morphological preservation than that previously obtained using tissue prepared with Lowicryl for immunogold detection of vesicular glutamate transporters (VGLUTs) and SNARE proteins[71], the tissue was perfusion-fixed with a mixture of 2.5% glutaraldehyde and 1% formaldehyde, and postfixed with 1% osmium tetroxide before being embedded in Durcupan (Fluka AG, Switzerland). Micrograph courtesy of V. Gundersen, Anatomical Institute, University of Oslo, Norway (unpublished observations).

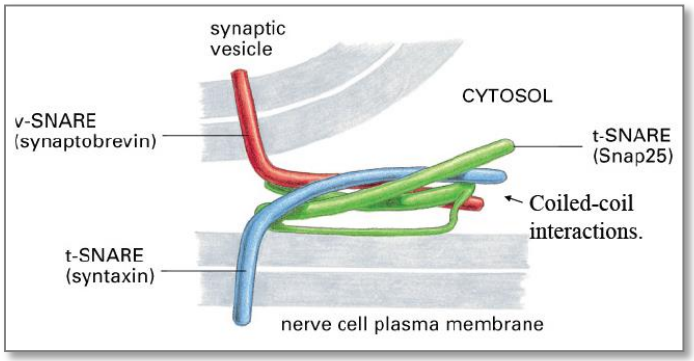
Precise intracellular machinery involved in the release of glutamate, D-serine, and ATP from astrocytes

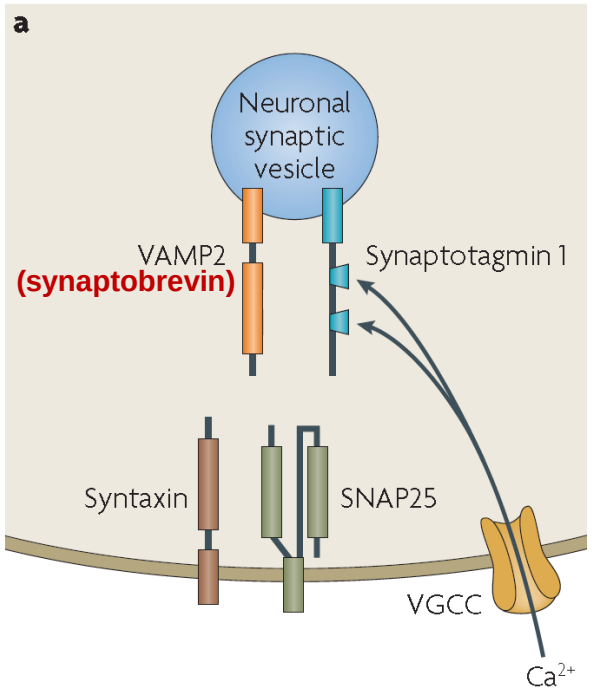
Harada et al., 2016, Frontiers in Neurosci.
doi: 10.3389/fnins.2015.00499

Glutamate and D-serine are taken up into **synaptic-like vesicles** through (1) VGLUT and (2) vesicular D-serine transporters (VSERT), respectively. These synaptic-like vesicles fuse to the plasma membrane, mediated by SNARE proteins including **VAMP2 or VAMP3**, in response to [Ca2+]*i* increase. In contrast, ATP is released through **secretory lysosomes**. Storage of ATP into secretory lysosomes is achieved by (3) VNUT. Through the interaction of SNARE proteins including **TI-VAMP**, ATP-containing secretory lysosomes are Ca2+-dependently exocytosed. Moreover, the existence of **other release mechanisms** has been discovered: (4) reverse operation of plasma membrane glutamate transporters, (5) cell swelling-induced anion transporter (VRAC) opening, (6) release via P2X7 receptors, and (7) gap junction channels (hemichannels) on the cell surface of astrocytes.



Comparison of Ca^{2+}-dependent exocytosis in neurons and astrocytes: SNAREs proteins involved





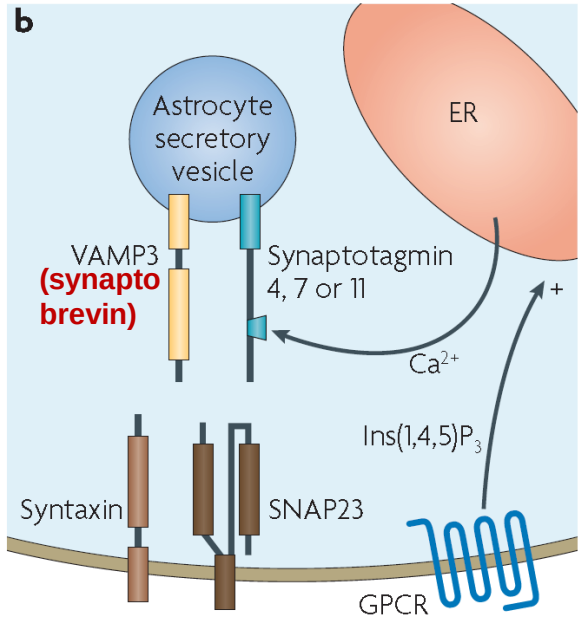
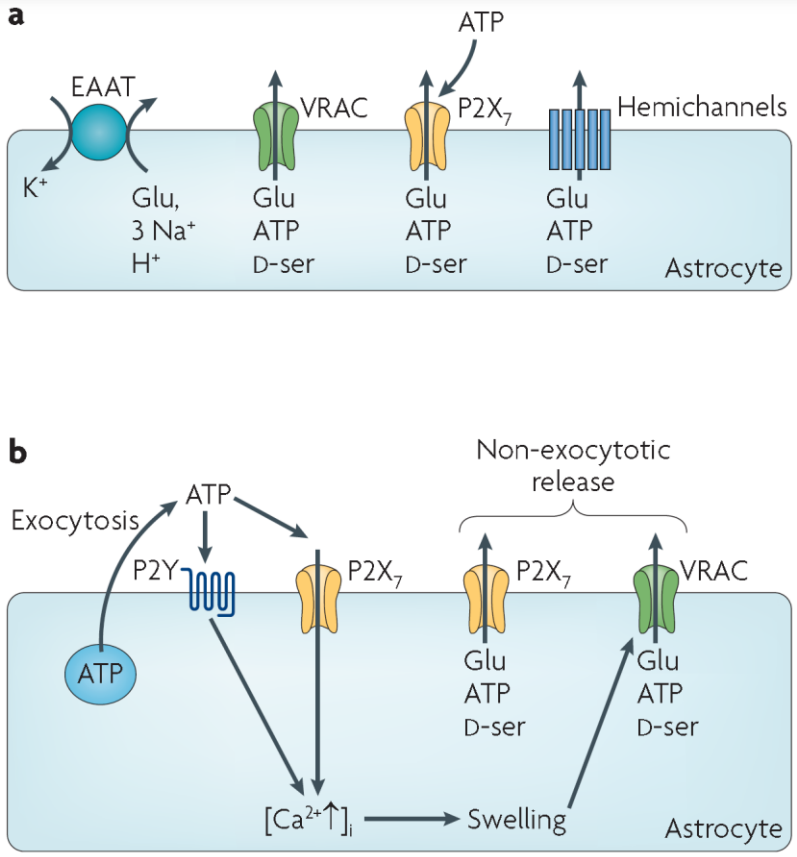
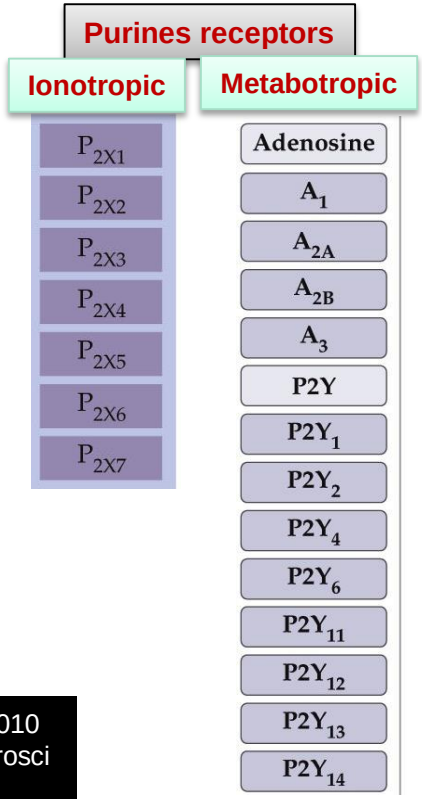


Figure 3 | **Proteins proposed to mediate exocytosis from neurons and astrocytes.** **a** | For the formation of a functional SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) complex that mediates vesicle fusion, syntaxin and synaptosomal-associated protein 25 (SNAP25) at the neuronal plasma membrane bind to vesicle-associated membrane protein 2 (VAMP2; also known as synaptobrevin 2). This is regulated by Ca^{2+}, normally entering from outside the cell through voltage-gated Ca^{2+} channels (VGCCs), binding to two sites of the Ca^{2+} sensor synaptotagmin 1. **b** | In astrocytes, SNAP23 has an analogous role to neuronal SNAP25, and VAMP3 (also known as cellubrevin) has an analogous role to VAMP2. The Ca^{2+} sensor may be synaptotagmin 4 or synaptotagmin 11 (each of which has one Ca^{2+}-binding site, as shown) or synaptotagmin 7 (which has two Ca^{2+}-binding sites). Activation of G protein-coupled receptors (GPCRs) at the plasma membrane generates inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$), which binds to its receptor on the endoplasmic reticulum (ER) and triggers the release of Ca^{2+} from the ER, resulting in vesicle fusion. Other proteins that are involved, including the monomeric G protein RAB, the syntaxin-binding protein MUNC18 and complexin, are not shown.

Hamilton & Attwell, 2010
Nature Reviews Neurosci
doi:10.1038/nrn2803

Non-exocytotic and hybrid release mechanisms for gliotransmitters





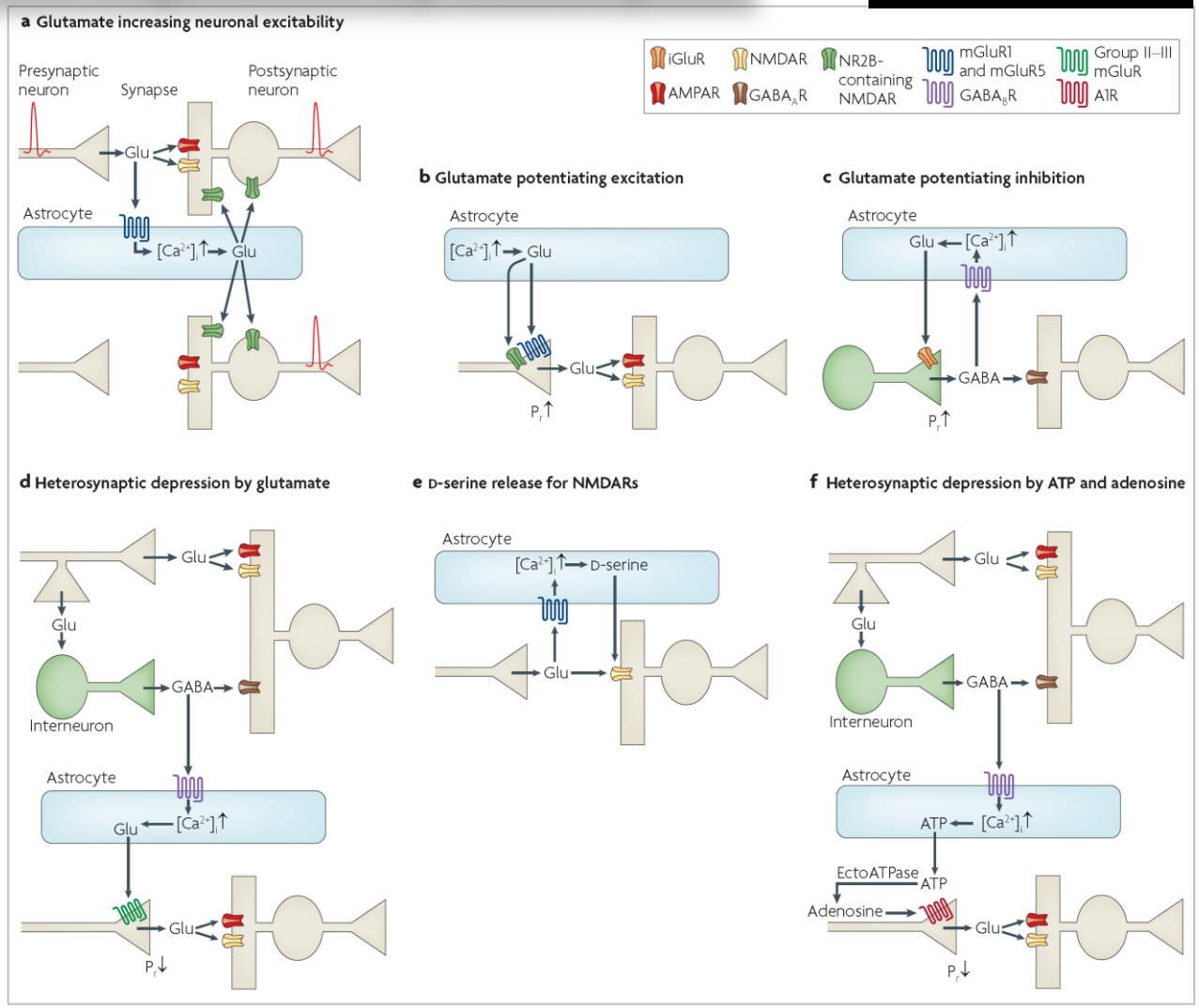
Hamilton & Attwell, 2010
Nature Reviews Neurosci
doi:10.1038/nrn2803

a | **Non-exocytotic transmitter release** can occur by reversal of plasma membrane glutamate (Glu) transporters (excitatory amino-acid transporters (EAATs)), or (for glutamate, ATP and d-serine (d-ser)) by efflux through volume-regulated anion channels (VRACs), ATP-gated P2X purinoceptor 7 (P2X7) receptor channels or gap junctional hemichannels formed by connexins or pannexins.

b | **Hybrid release mechanisms** might occur if exocytotic release of ATP activates P2X7 and P2Y receptors. This will allow non-exocytotic transmitter release through P2X7 receptors and VRACs. VRACs are activated by cell swelling produced by the increase in intracellular Ca2+ concentration ([Ca2+]i) generated by the P2X7 or P2Y receptors. These non-exocytotic release mechanisms depend on the initial exocytosis of ATP, and so will be inhibited by preventing the [Ca2+]i increase.

Synaptic transmission regulation by astrocytes

Hamilton & Attwell, 2010
Nature Reviews Neurosci
doi:10.1038/nrn2803



a | Modulation of neuronal excitability and synchrony by glutamate (Glu) release from astrocytes. Stimulating the Schaffer collateral input to area CA1 (top left) evokes glutamate release that triggers fast synaptic currents in CA1 pyramidal cells (top right), as well as an increase in [Ca2+]i in astrocytes mediated by type 1 and type 5 mGluRs. This releases glutamate from the astrocytes, which activates extrasynaptic NR2B subunit-containing NMDARs (shown in green) in nearby pyramidal cells, generating slow inward currents that enhance excitability and synchronize firing of these neurons (the two neurons on the right).

b | Glutamate release from astrocytes increases presynaptic glutamate release from neurons. A rise of astrocyte [Ca2+]i leads to glutamate release, which activates presynaptic NR2B subunit-containing NMDARs or group I mGluRs, increasing the probability of transmitter release (Pr).

c | Glutamate release from astrocytes, triggered by GABA activating astrocyte GABAB receptors (GABABRs), **increases presynaptic GABA release.**

d | Heterosynaptic depression mediated by astrocyte glutamate release. Stimulating the Schaffer collaterals evokes GABA release from hippocampal interneurons, which activates GABAB receptors on astrocytes. The resulting [Ca2+]i increase releases glutamate, which acts on presynaptic group II–III mGluRs to suppress glutamate release from other afferents.

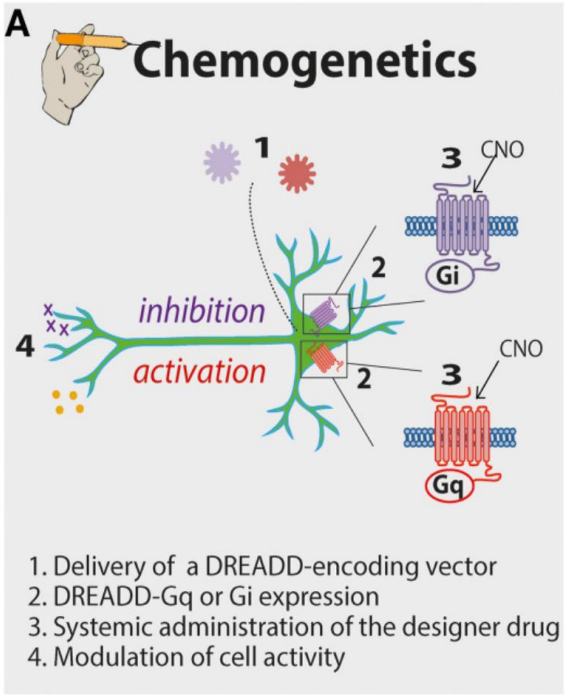
e | NMDAR activation regulated by Ca2+-dependent release of d-serine from astrocytes. d-serine activates neuronal NMDARs by binding to the NR1 subunit, thus controlling synaptic plasticity. In cultured cells, the [Ca2+]i increase that controls d-serine release has been shown to occur in response to the activation of astrocyte mGluRs, AMPARs or kainate receptors by glutamate.

f | Heterosynaptic depression mediated by astrocyte ATP release. Stimulating the Schaffer collaterals evokes GABA release from hippocampal interneurons, which activates GABAB receptors on astrocytes. The resulting increase in [Ca2+]i releases ATP, which is degraded to adenosine by extracellular ATPases (EctoATPases). The adenosine activates presynaptic A1 receptors (A1Rs) and suppresses glutamate release from other afferents. Note the similarity to **d**.

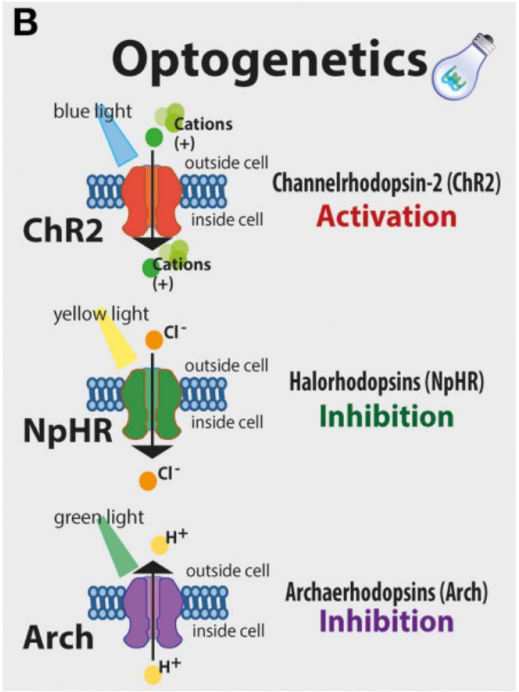
Optogenetic and Chemogenetic Approaches for Studying Astrocytes and Gliotransmitters

Aviello & D'Agostino, 2016
Frontiers in Pharmacology
doi: 10.3389/fphar.2016.00043

Optogenetics and chemogenetics allow functional manipulations both in vitro and in vivo to examine causal relationships between cellular changes and functional outcomes. These techniques are based on **genetically encoded effector molecules that respond exclusively to exogenous stimuli, such as a certain wavelength of light or a synthetic ligand**. Activation of effector molecules provokes diverse intracellular changes, such as an influx or efflux of ions, depolarization or hyperpolarization of membranes, and activation of intracellular signaling cascades. Optogenetics and chemogenetics have been applied mainly to the study of neuronal circuits, but their use in studying non-neuronal cells, in particular **astrocytes**, has been gradually increasing.



In panel **(A) t**he principles of the chemogenetic technology are schematized: these include the delivery of a **DREADD** (designer receptors exclusively activated by designer drugs) encoding vector, the expression of the designer receptor in the cell population of interest, and the modulation of this receptor by a designer drug. In panel **(B)** the principles of the optogenetic technology and the mechanisms by which commonly used **opsins** modulate cell activity are schematized and simplified.



Bang et al., 2016
https://doi.org/10.5607/en.2016.25.5.205
Exp Neurobiol. 2016 Oct;25(5):205-221.

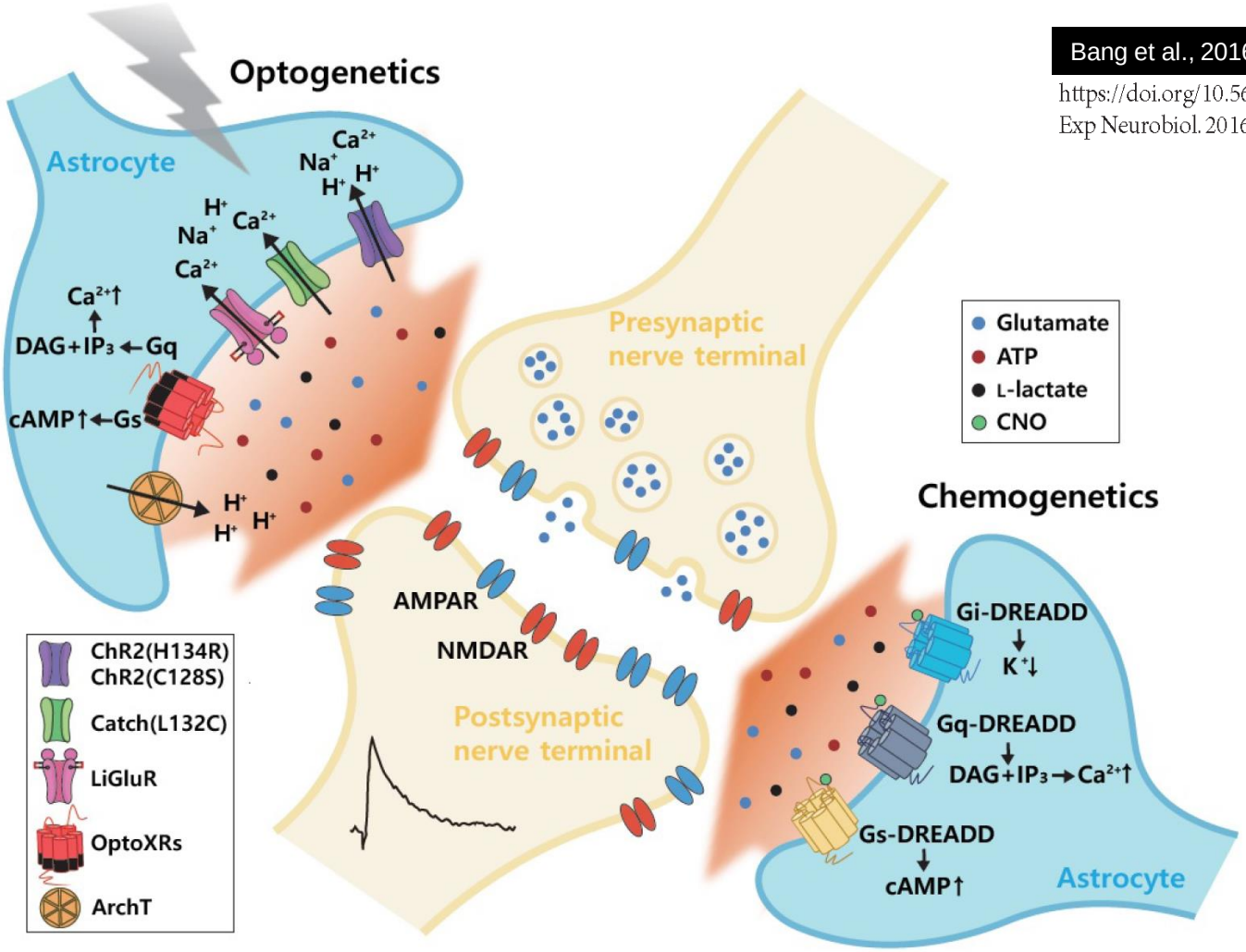
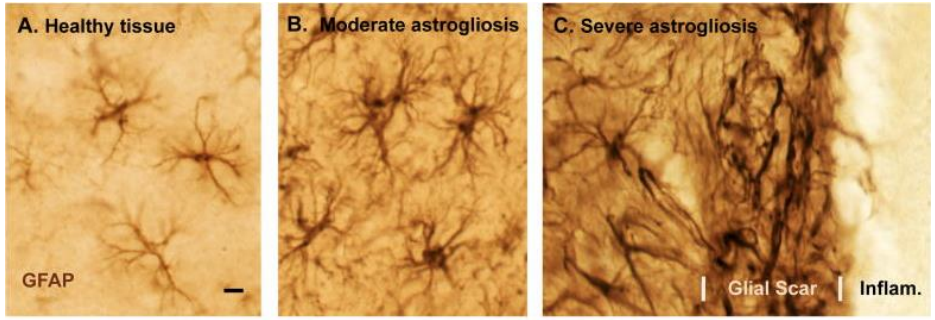


Fig. 1. Optogenetic and chemogenetic stimulation of astrocytes. A variety of genetically encoded effector molecules for optogenetics (left) and chemogenetics (right) have been employed to manipulate intracellular ionic concentrations (H^+, Na^+, Ca^{2+}, K^+) and signaling cascades (Gq, Gs, DAG, IP_3, cAMP) in astrocytes. Intracellular changes such as cytosolic calcium increase and acidification, in turn, evoke release of signaling molecules, so-called gliotransmitters (glutamate, ATP, L-lactate), from astrocytes, which modulate excitability as well as synaptic transmission of neighboring neurons. Optogenetic effectors can be activated by specific wavelengths of photostimulation, and chemogenetic effectors can be activated by synthetic ligands, such as CNO. ChR2, channelrhodopsin-2; CatCh, calcium translocating channelrhodopsin; LiGluR, light-gated ionotropic glutamate receptor 6; ArchT, archaerhodopsin; OptoXRs, light-driven chimeric G protein-coupled receptors; NMDAR, N-methyl-D-aspartate receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; Gi-DREADD, Gi-coupled designer receptors exclusively activated by designer drugs; Gq-DREADD, Gq-coupled DREADD; Gs-DREADD, Gs-coupled DREADD; CNO, clozapine-N-oxide; ATP, adenosine triphosphate; IP_3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; cAMP, cyclic adenosine monophosphate.

Astrocytosis

- Astrocytosis/gliosis: response of astrocytes to many forms of injury: trauma, inflammation, MS, infection, neurodegeneration
- Classical description of gliosis is hypertrophy, glial filament production +/- proliferation.
- Reality: there must be many distinct forms of astrocyte activation; hundreds or thousands of distinct changes in gene expression



Different grades of reactive gliosis

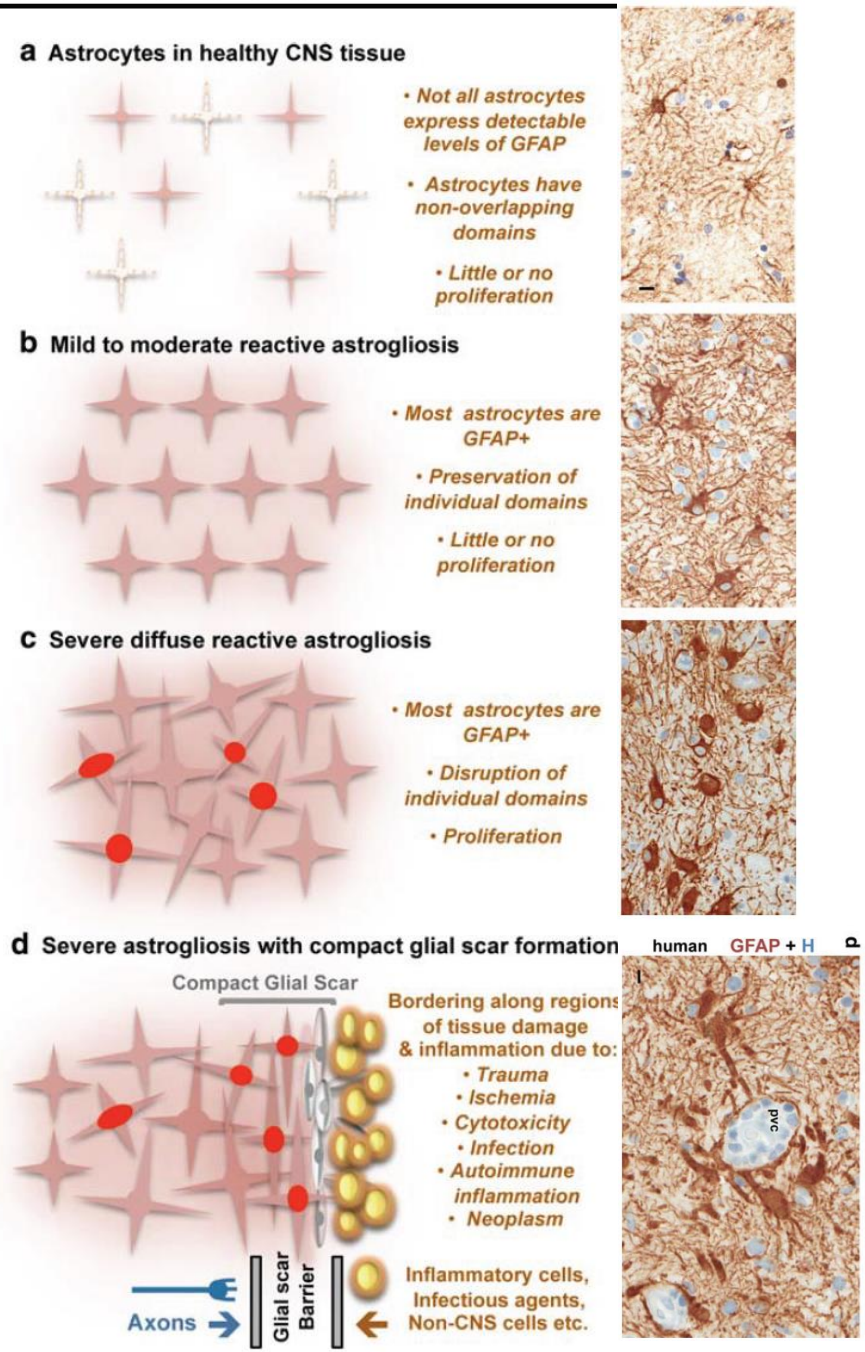


Fig. 4 Schematic representations that summarize different gradations of reactive astrogliosis. **a** Astrocytes in healthy CNS tissue. **b** Mild to moderate reactive astrogliosis comprises variable changes in molecular expression and functional activity together with variable degrees of cellular hypertrophy. Such changes occur after mild trauma or at sites distant from a more severe injury, or after moderate metabolic or molecular insults or milder infections or inflammatory activation. These changes vary with insult severity, involve little anatomical overlap of the processes of neighboring astrocytes and exhibit the potential for structural resolution if the triggering insult is removed or resolves. **c** Severe diffuse reactive astrogliosis includes changes in molecular expression, functional activity and cellular hypertrophy, as well newly proliferated astrocytes (with *red* nuclei in figure), disrupting astrocyte domains and causing long-lasting reorganization of tissue architecture. Such changes are found in areas surrounding severe focal lesions, infections or areas responding to chronic neurodegenerative triggers. **d** Severe reactive astrogliosis with compact glial scar formation occurs along borders to areas of overt tissue damage and inflammation, and includes newly proliferated astrocytes (with *red* nuclei in figure) and other cell types (*gray* in figure) such as fibromeningeal cells and other glia, as well as deposition of dense collagenous extracellular matrix. In the compact glial scar, astrocytes have densely overlapping processes. Mature glial scars tend to persist for long periods and act as barriers not only to axon regeneration but also to inflammatory cells, infectious agents, and non-CNS cells in a manner that protects healthy tissue from nearby areas of intense inflammation

Sofroniew & Vinters, 2010
Acta Neuropathol
DOI 10.1007/s00401-009-0619-8

Astrogliosis in cerebro-vascular deseases

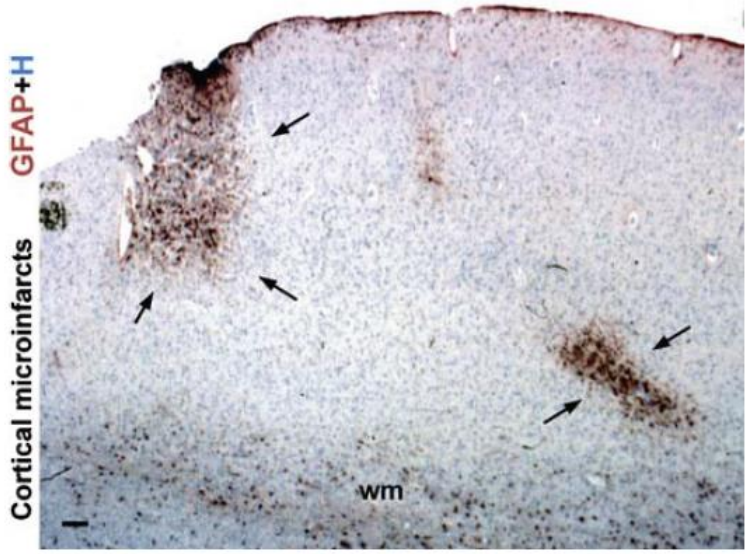


Fig. 5 Reactive astrogliosis demarcates cerebral microinfarcts. Survey image of cerebral cortex of an elderly individual showing microinfarcts (*arrows*) highlighted by dense clusters of prominently reactive astrocytes that stain intensely for GFAP. Fibrous astrocytes within subcortical white matter (*wm*) exhibit GFAP staining, whereas GFAP is not detectable in most protoplasmic gray matter astrocytes remote from the lesions in this specimen. *H* haematoxylin counterstain. *Scale bar* 180 µm

Sofroniew & Vinters, 2010
Acta Neuropathol
DOI 10.1007/s00401-009-0619-8

Astrogliosis in Alzheimer desease

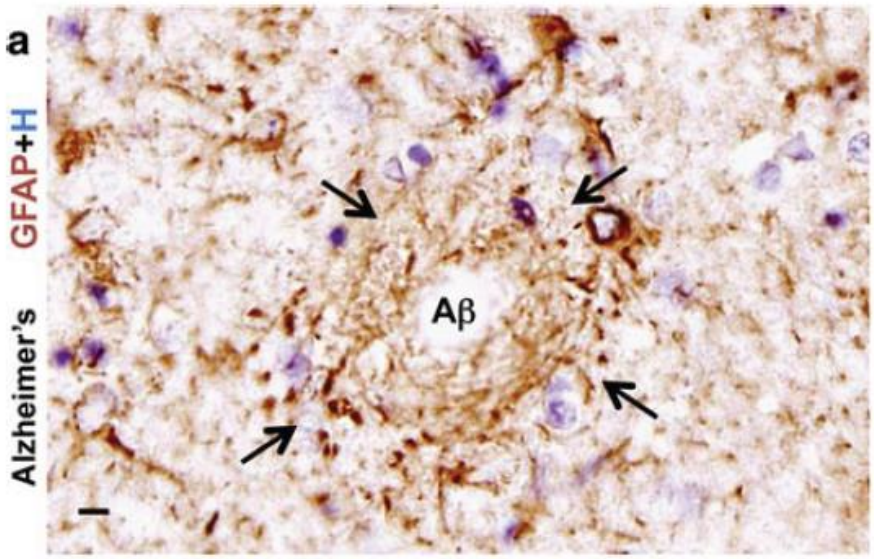
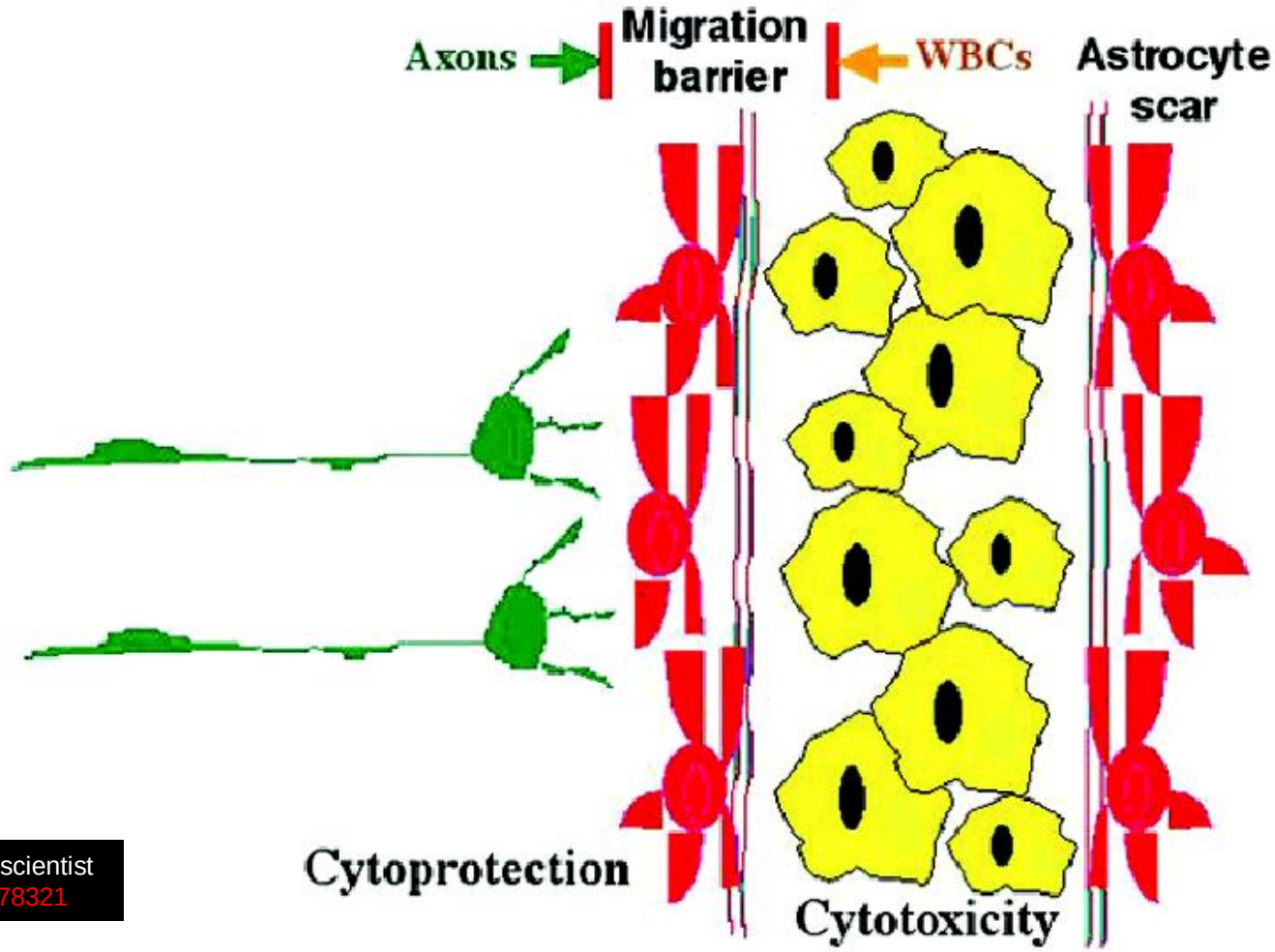


Fig. 7 Reactive astrogliosis in two degenerative diseases. **a** High magnification image of autopsy specimen from a person with longstanding Alzheimer's disease immunohistochemically stained for GFAP. Section of cerebral cortex shows an amyloid senile plaque with a pale unstained center (Aβ) ringed by dense layers of reactive astrocytic processes (*arrows*) that circumferentially surround the plaque as if forming a scar-like barrier around it. **b** High magnifi-

Astrocytic scar: good or bad?



Sofroniew, 2005, The Neuroscientist
DOI: 10.1177/1073858405278321

Fig. 4. Schematic of barrier functions of scar-forming reactive astrocytes. Scar tissue organized by reactive astrocytes walls off areas of compromised tissue. Within the walled-off area, a robust inflammatory reaction occurs with the release of potent cytotoxic agents targeted at potential invading microorganisms, but that also sacrifices local neural cells. Outside of and immediately adjacent to the astrocyte scar, inflammation is minimal and cytoprotective mechanisms are active. Although the astrocyte scar may serve primarily as a migration barrier that keeps inflammatory white blood cells (WBCs) from invading adjacent healthy tissue, the redundancy of migratory guidance cues among neurons and leukocytes may account for the inhibition of axon regeneration by this barrier.

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.

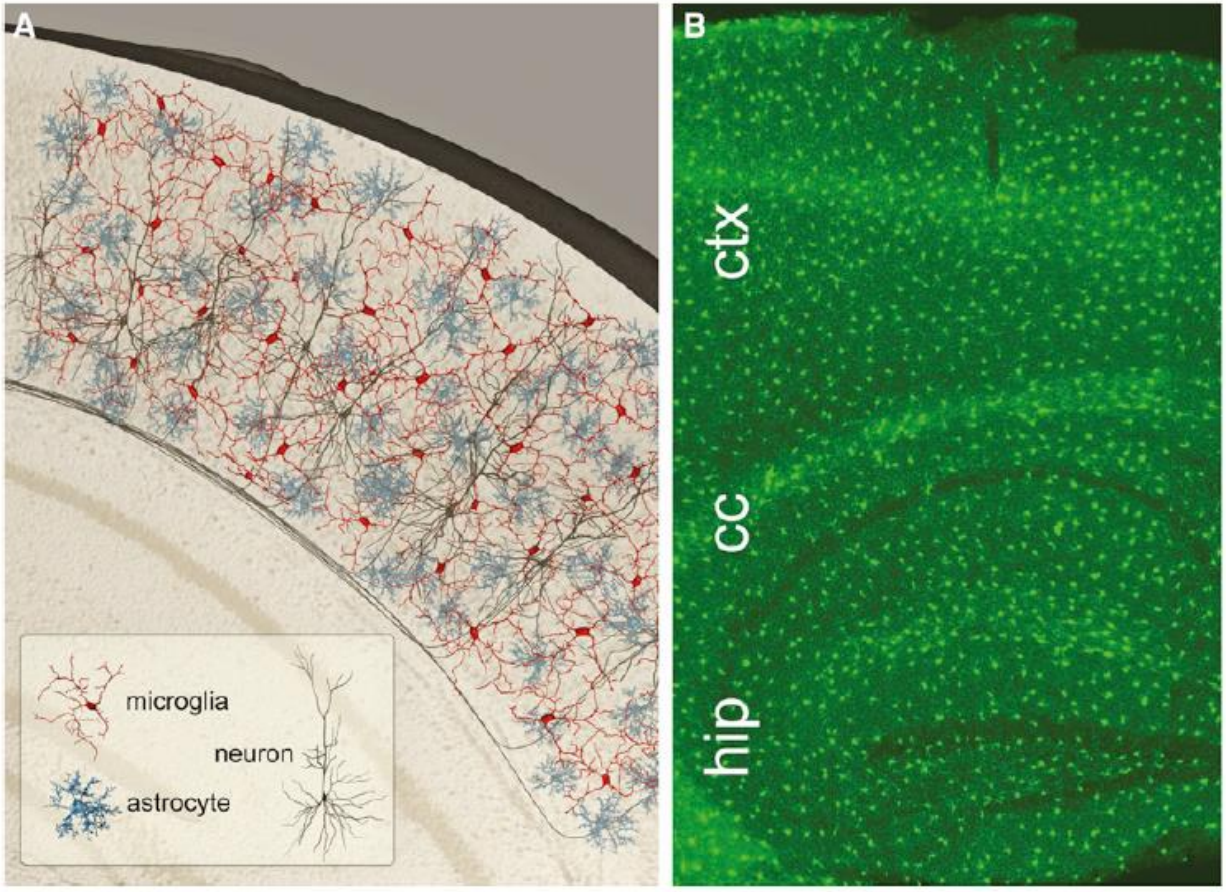


Figure 1. Uniform Distribution of Microglia in the Central Nervous System

(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).

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.

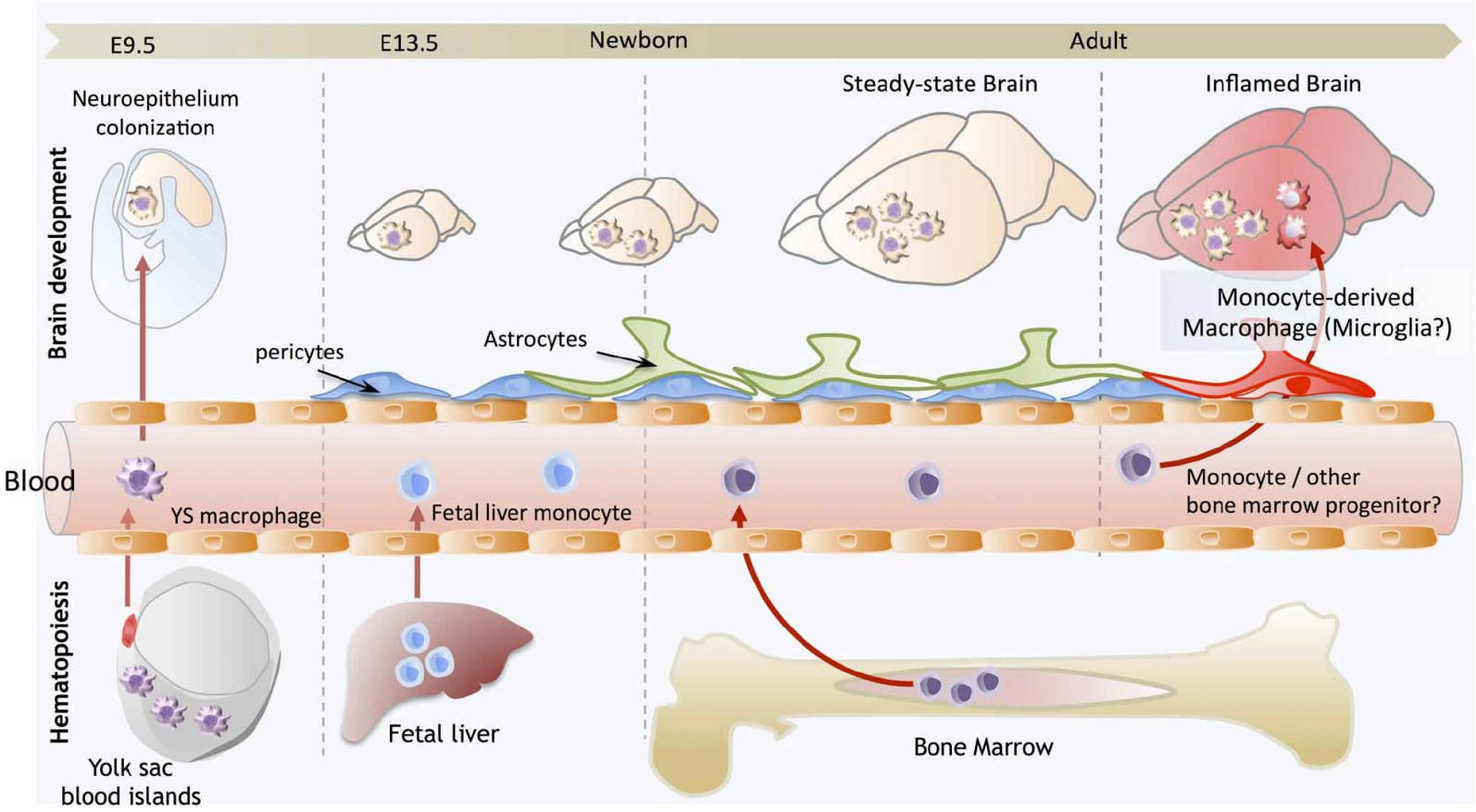


FIGURE 1 | Brain development and microglial homeostasis. Primitive macrophages exit the yolk sac blood islands at the onset of circulation and colonize the neuroepithelium from E9.5 to give rise to microglia. The blood brain barrier starts to form from E13.5 and may isolate the developing brain from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

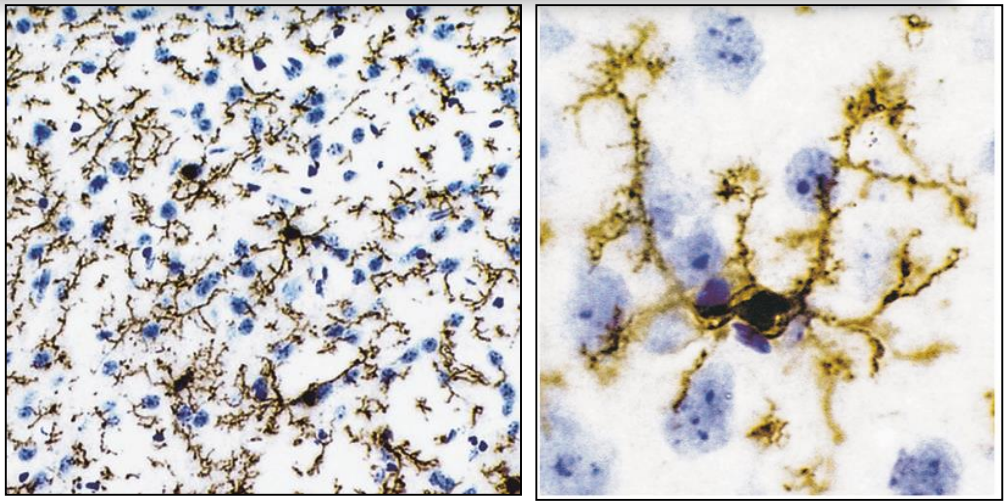


Figure 4–19 Large numbers of microglia reside in the mammalian central nervous system. The micrograph on the left shows microglia in the cerebral cortex of an adult mouse (in **brown**, immunocytochemistry). The **blue** spots are the nuclei of nonmicroglial cells. The microglial cells have fine, lacy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

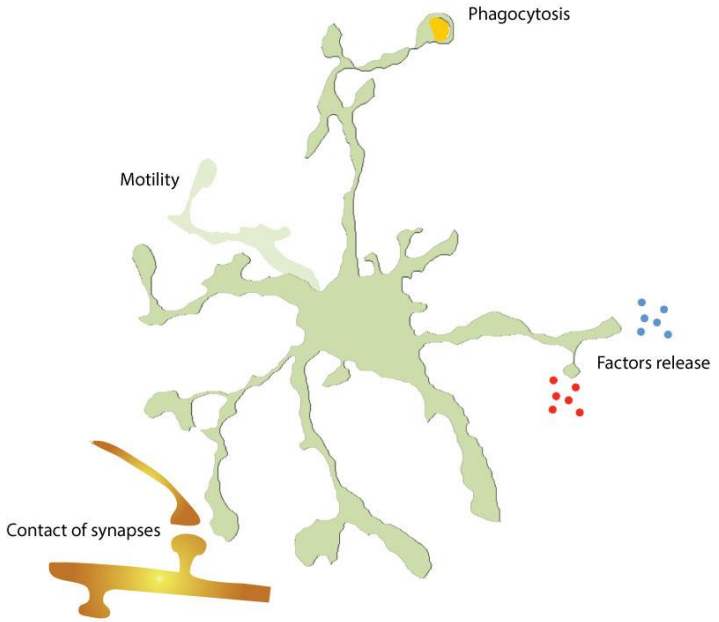


Figure 2.6 Scheme of the different functions of microglia.
Microglia (green) constantly move their processes to scan the brain parenchyma. During their movements they contact synapses and neuronal dendrites (orange), as well other brain cells. They can control brain activity and surrounding cells' fate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

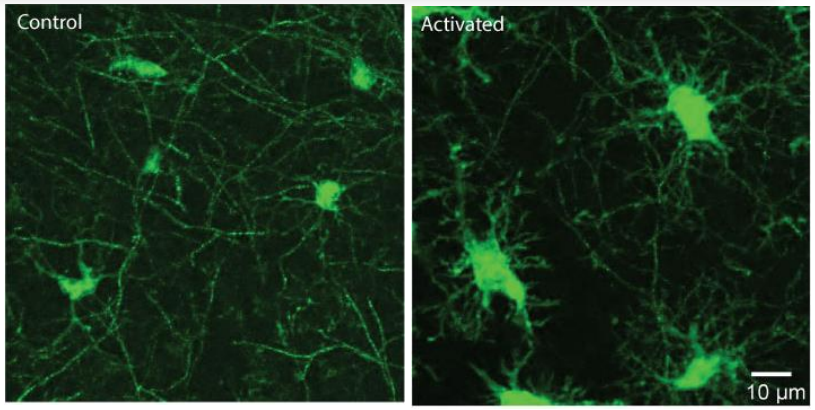


Figure 2.7 Microglia change properties after activation. The images show an example of morphological changes of microglia 48 hours after activation induced by *status epilepticus*. In control conditions (**a**) microglial cells have a small body with long and ramified processes. (**b**) In contrast,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

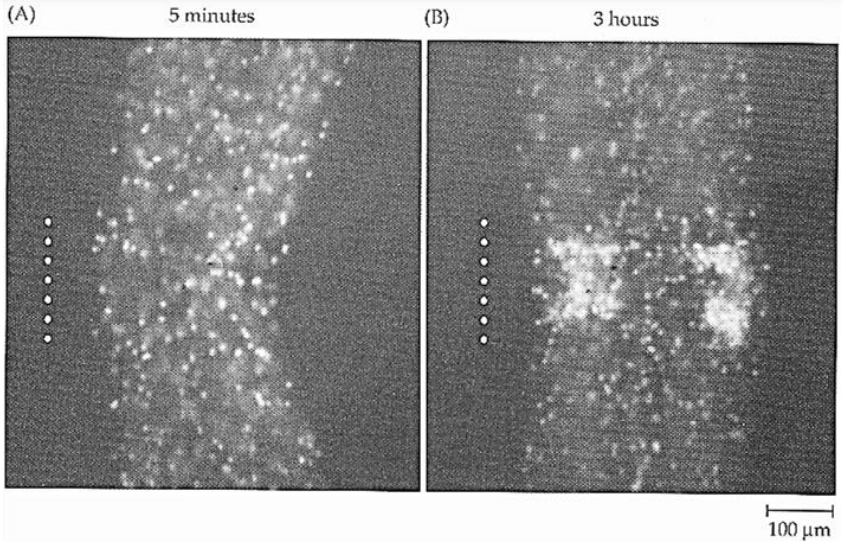
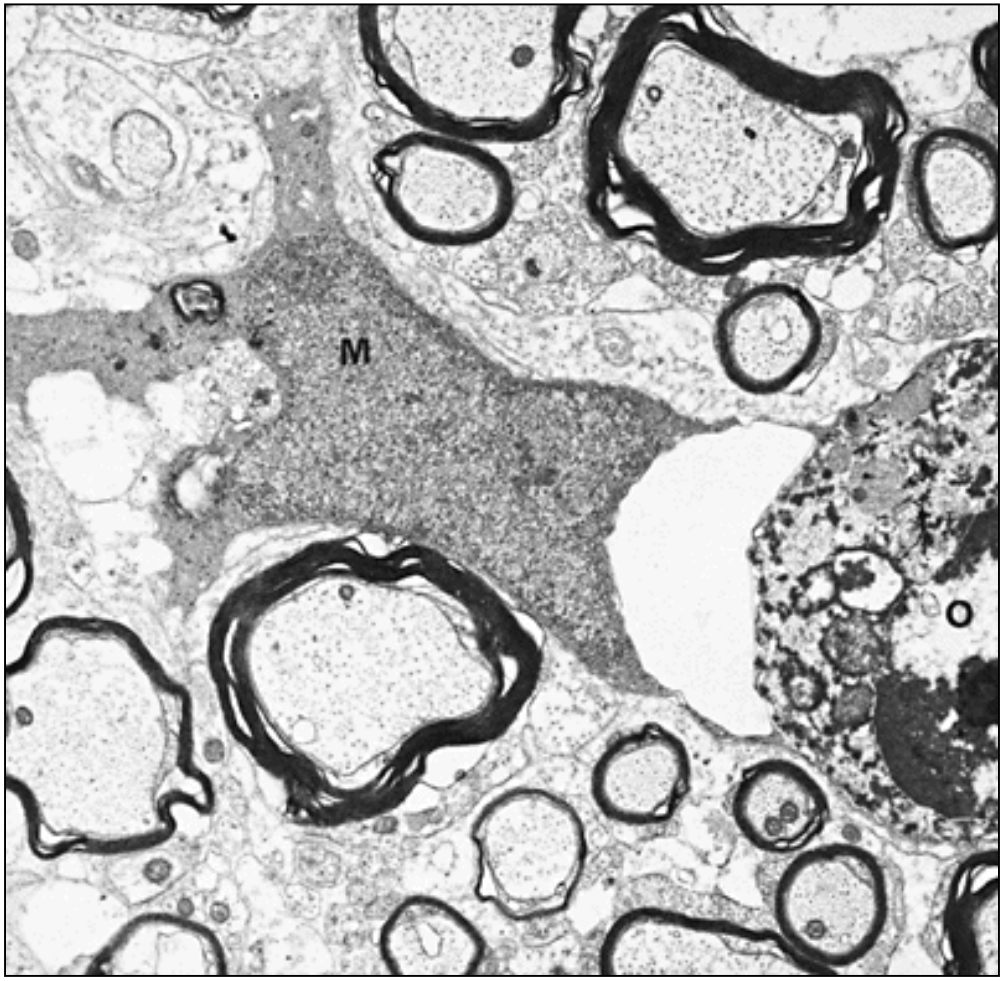


FIGURE 8.11 Migration of Microglial Cells in Injured CNS. (A) Microglia in the leech CNS were stained with a fluorescent nuclear dye (Hoechst 33342). The bundle of axons linking ganglia had been crushed 5 minutes earlier. The extent of the crush is indicated by the dotted line. The nuclei of microglial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



How many functional roles of MICROGLIA?

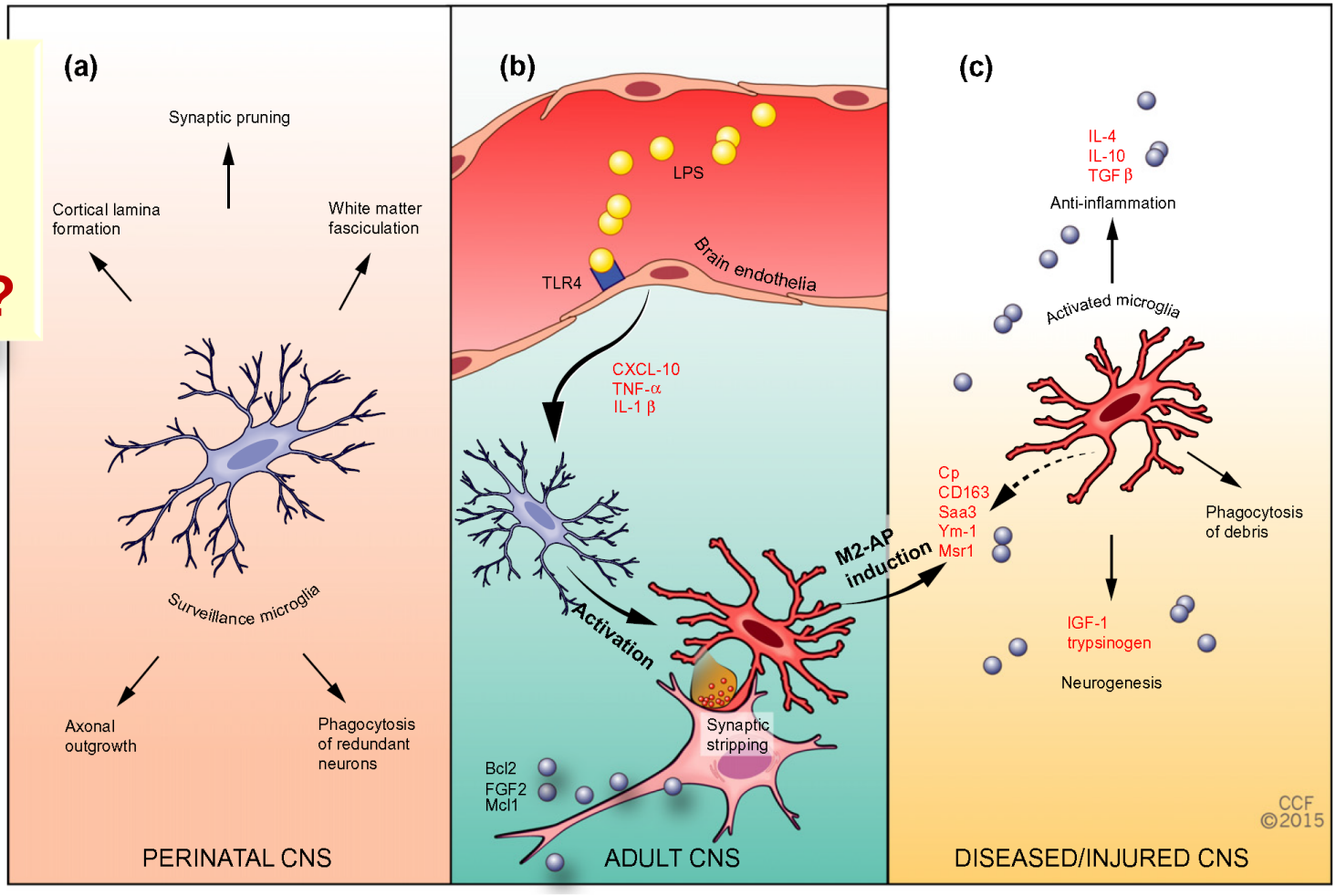


Fig. 1 Microglia contribute to CNS homeostasis and neuroprotection during development (a), adulthood (b), and CNS diseases (c). (a) Microglia maintain tissue homeostasis during brain development by pruning synapses or phagocytizing redundant neurons. They also participate in the proper formation of CNS 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 and hippocampal neurogenesis

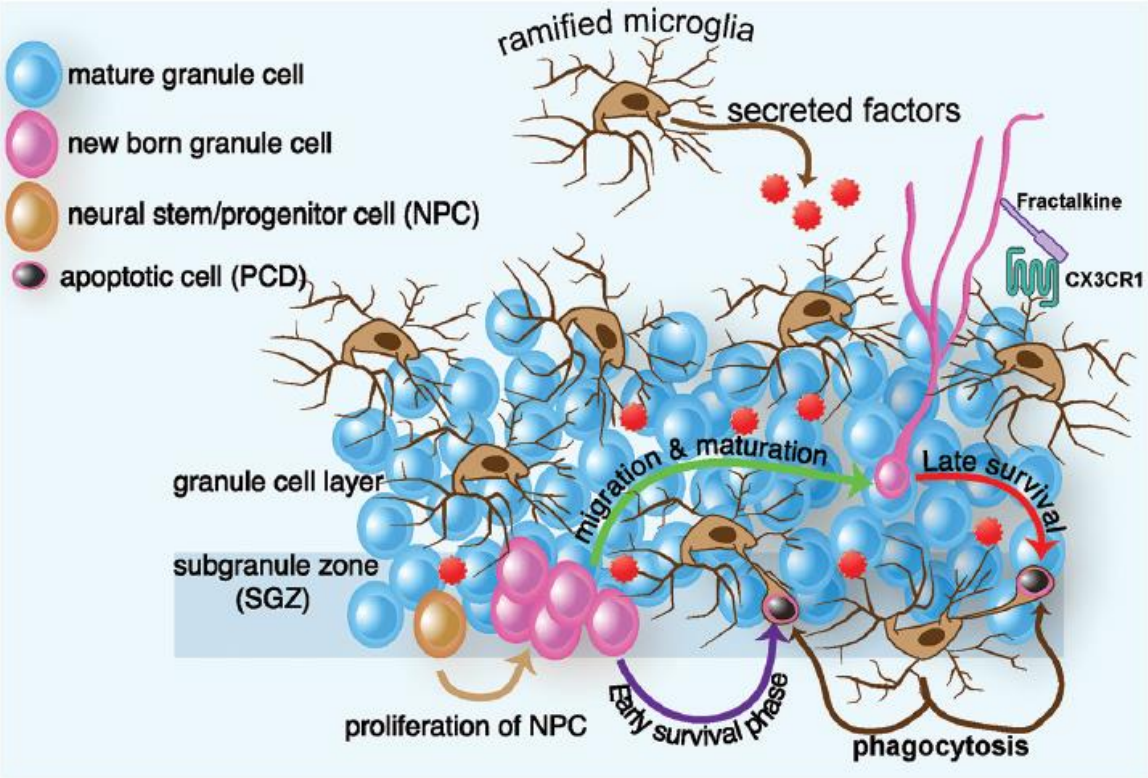


FIGURE 1 | Schematic diagram of ramified microglia and their effect on adult hippocampal neurogenesis. In intact brain, microglia regulate several steps of adult hippocampal neurogenesis. In the SGZ, progenitor cells migrate to the granule cell layer and differentiate into a neuronal phenotype, with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

Microglial cells can sense neuronal activity

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

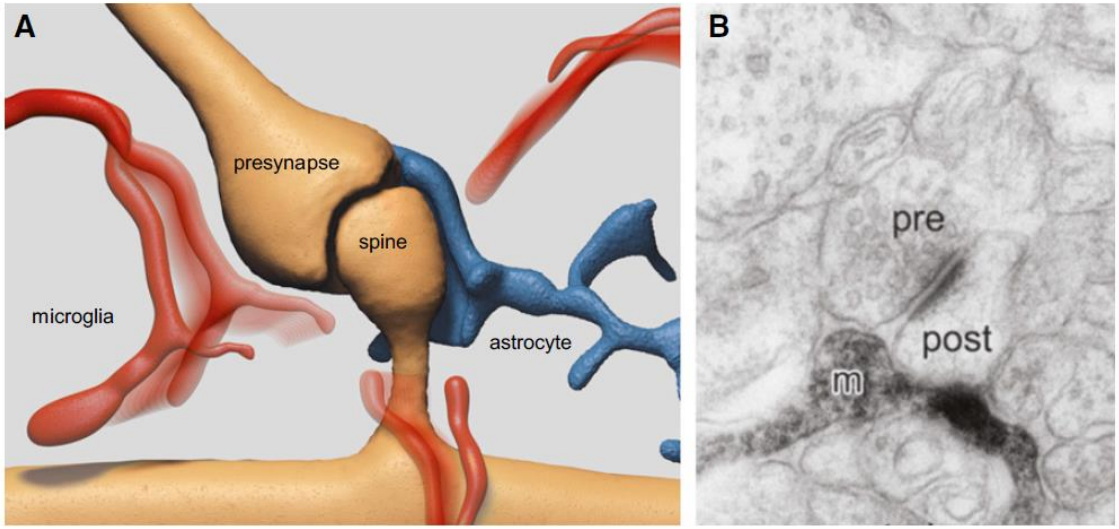


Figure 2. Dynamic Interaction of Microglial Processes with the Tripartite Synapse

(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

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

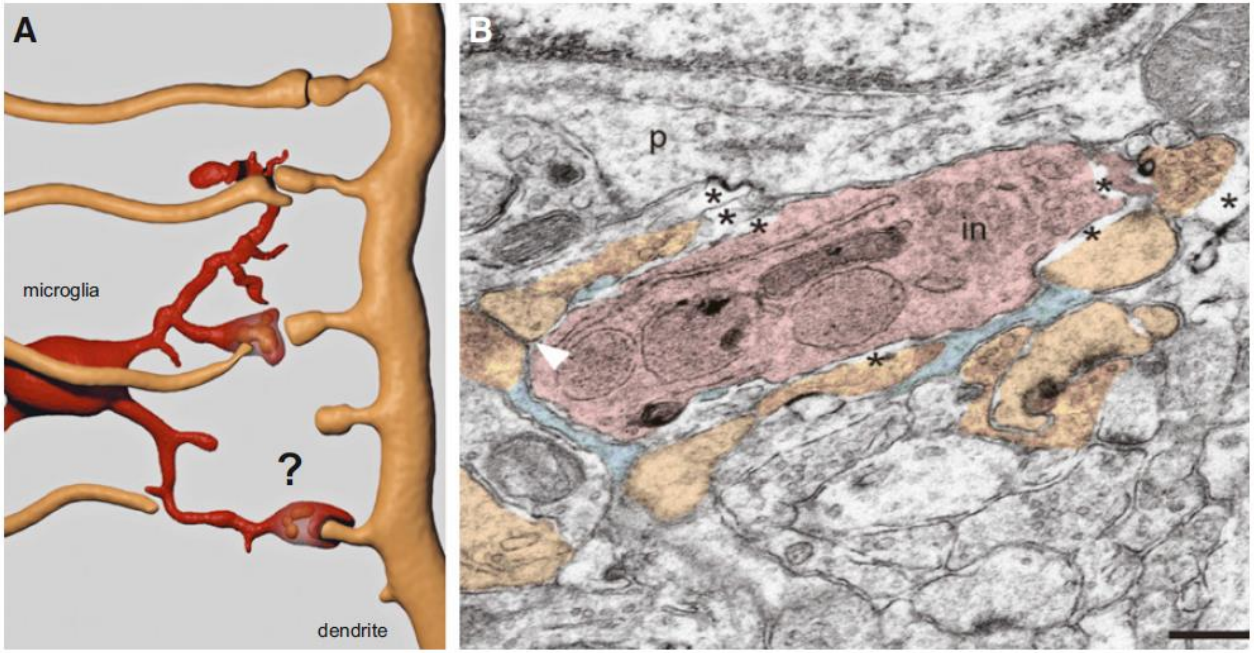


Figure 3. Synaptic Pruning by Microglial Processes

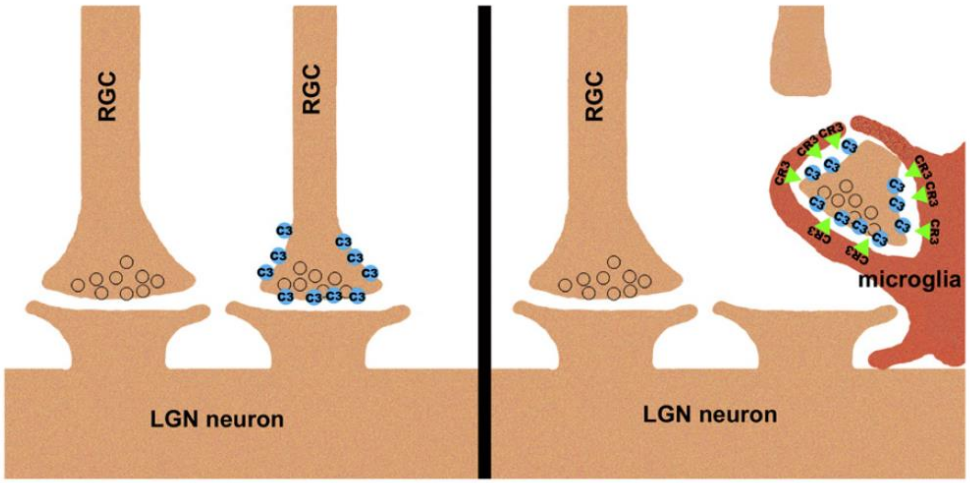
(A) The stability and maintenance of presynaptic terminals and postsynaptic spines is determined by microglia in a three-step process called synaptic pruning composed of contact, engulfment, and phagocytosis of presynaptic terminals. Whether dendritic spines are similarly removed by microglia is still 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 Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner

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



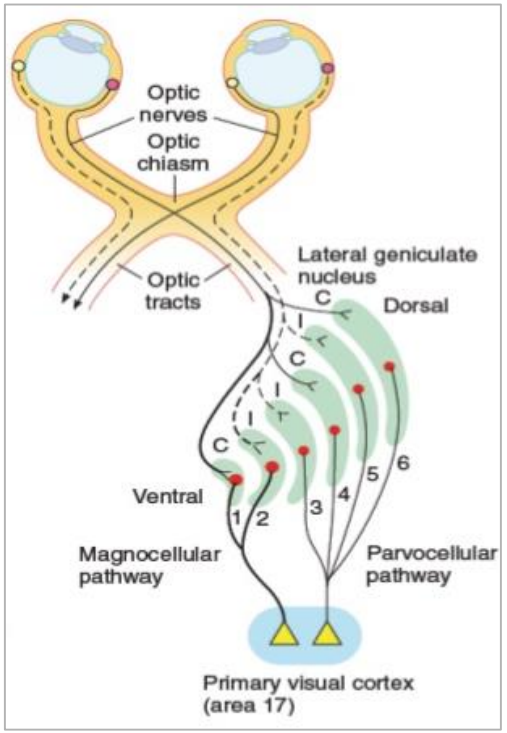


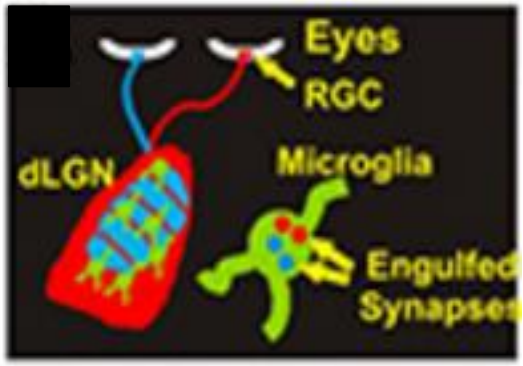
Figure 1. Microglia Phagocytose RGC Axon Material in a C3- and CR3-Dependent Manner

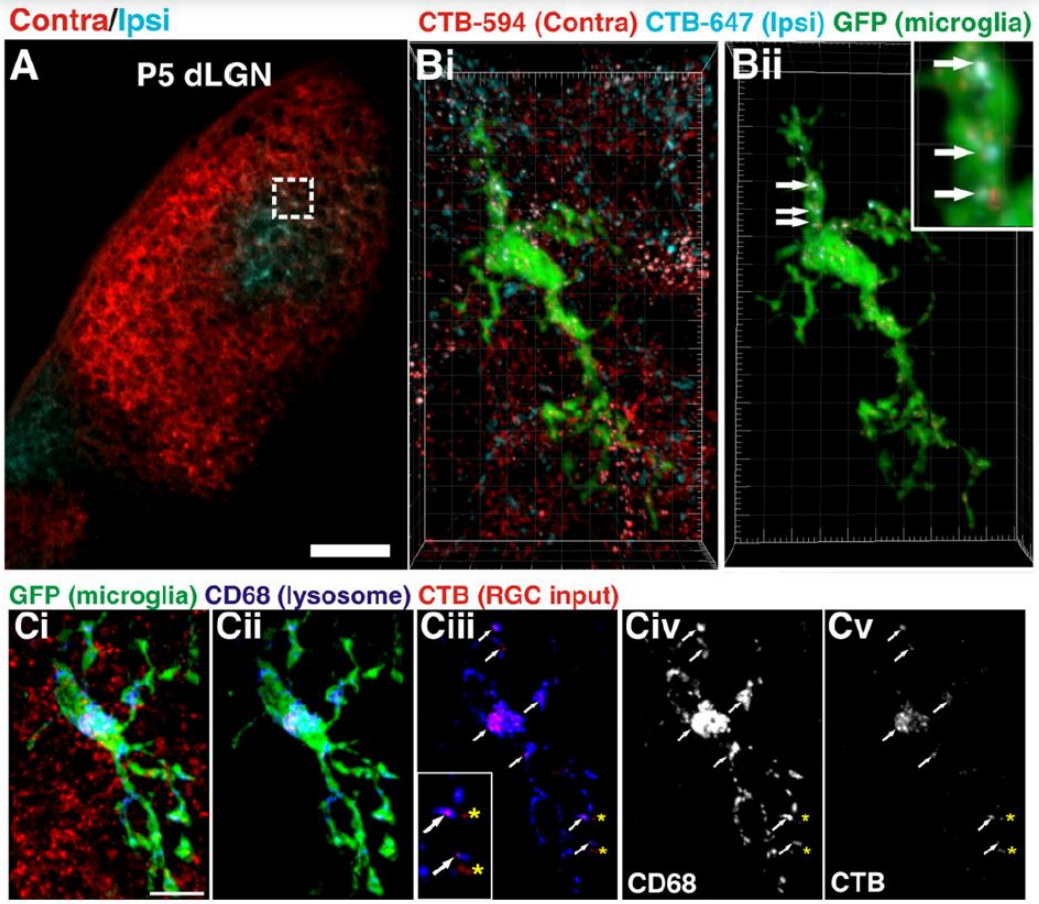
Proteins of the major histocompatibility complex class I (MHCI) and complement cascade (C1q and C3) are expressed in the developing brain and are necessary for normal pruning of **Retinal Ganglion Cells** (**RGC**) axons in the dorsal **Lateral Geniculate Nucleus** (**dLGN**). Schafer et al. demonstrate 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.





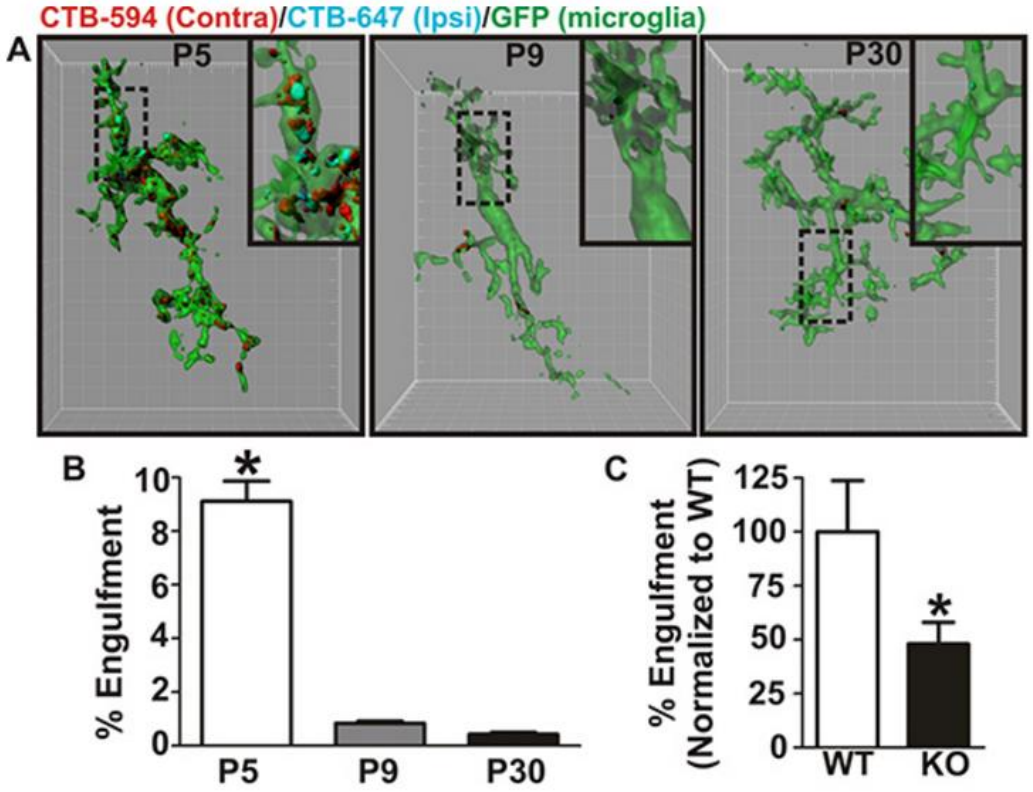
* Microglia were labeled using the CX3CR1+/GFP mouse line in which all microglia express EGFP under the control of fractalkine receptor, CX3CR1

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.

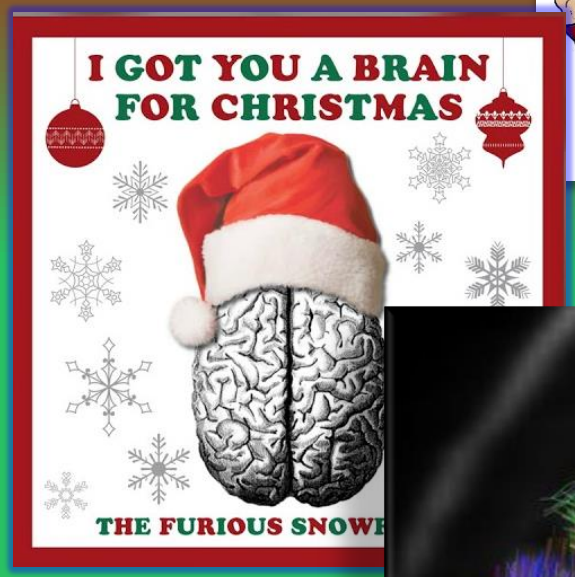
(Bi) A microglia (EGFP, green) sampled from the border region of ipsilateral (blue) and contralateral (red) projections (inset in A). (Bii) All CTB fluorescence outside the microglial volume has been subtracted revealing RGC inputs (red and blue) that have been engulfed (arrows, enlarged in inset). Grid 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.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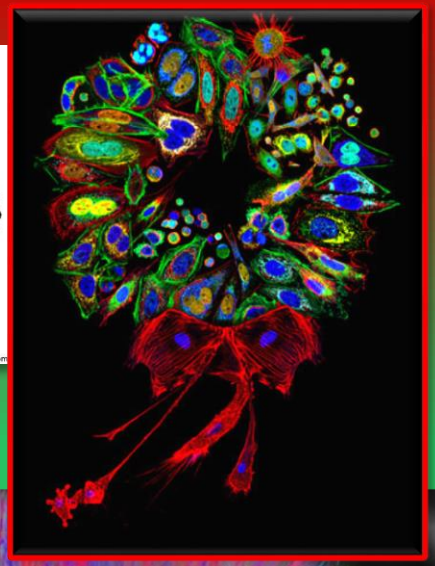


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