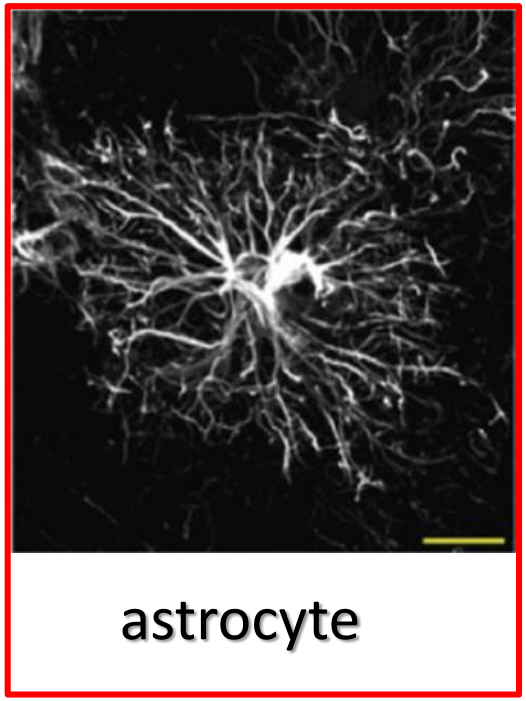
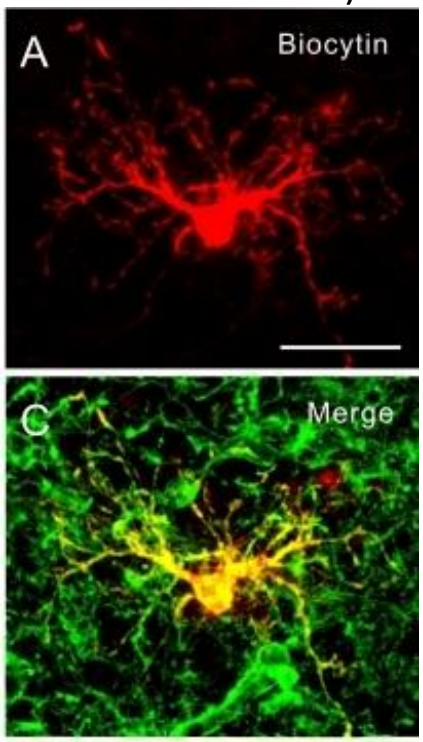
Non-myelinating glia in the CNS: ASTROCYTES, MICROGLIA, OPCs

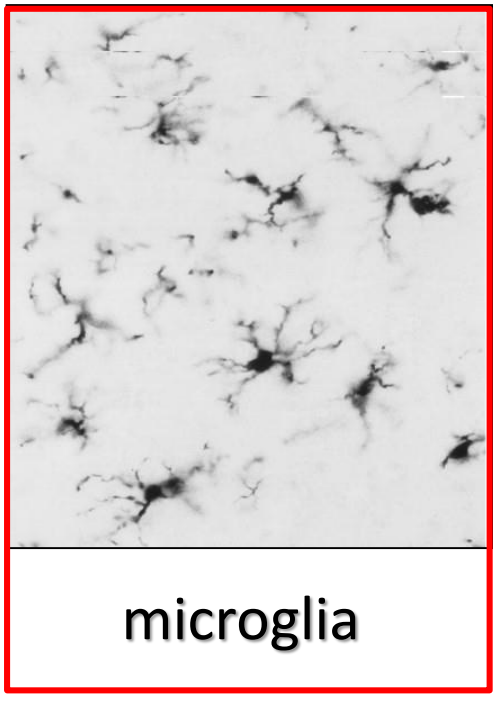


astrocyte

OPCs (Oligodendrocyte Precursor Cells)



Green: NG2 immunostaining

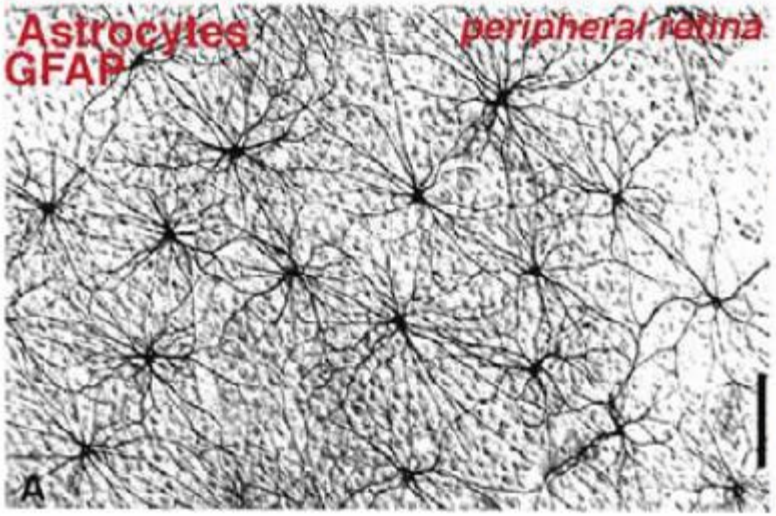


microglia

"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

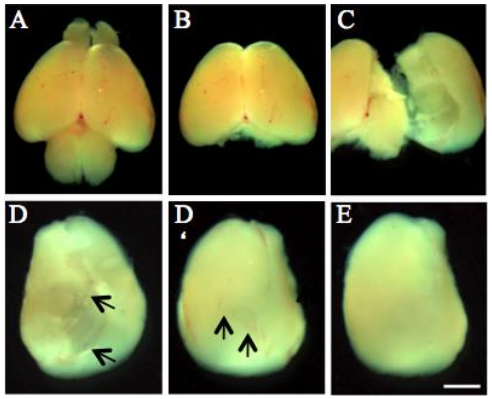
New role! → Gliotransmission: synaptic modulation

VIDEO: Isolation and Culture of Mouse Cortical Astrocytes

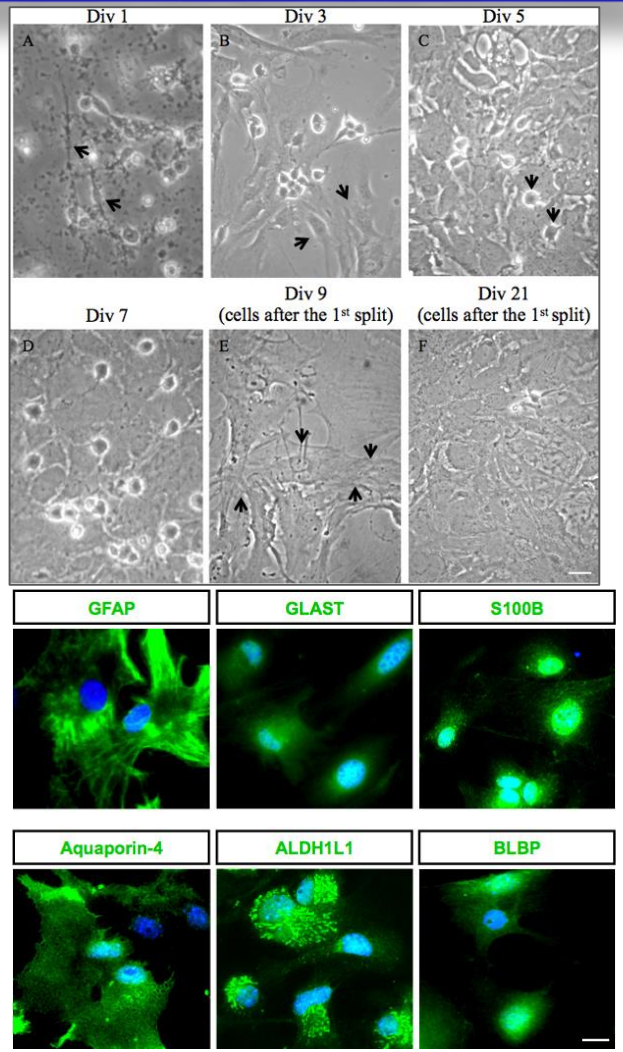
http://www.jove.com/video/50079/isolation-and-culture-of-mouse-cortical-astrocytes
https://www.jove.com/video/56092/culturing-vivo-like-murine-astrocytes-using-fast-simple-inexpensive

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

Protocol modified from McCarthy & de Vellis, 1980



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 time points 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.

https://www.jove.com/video/55108/monitoring-astrocyte-reactivity-proliferation-vitro-under-ischemic
https://www.jove.com/video/54757/the-indirect-neuron-astrocyte-coculture-assay-an-vitro-set-up-for

Isolation and Culture of Rat Cortical Astrocytes by immunopanning

Development of a Method for the Purification and Culture of Rodent Astrocytes

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DOI 10.1016/j.neuron.2011.07.022

The inability to purify and culture astrocytes has long hindered studies of their function. Whereas astrocyte progenitor cells can be cultured from neonatal brain, culture of mature astrocytes from postnatal brain has not been possible. Here, we report a new method to prospectively purify astrocytes by immunopanning. These astrocytes undergo

In this paper, we describe a new immunopanning method for prospectively isolating astrocytes from rodent CNS tissue. We have successfully isolated astrocytes from P1–P18 rats. Unlike the previous McCarthy and de Vellis method of astrocyte preparation, where cells were prepared by a series of steps extending over a week, we selected the astrocytes directly in a rapid isolation procedure that was completed in 1 day. We also report the development of a defined, serum-free medium that enables the survival of the purified astrocytes in long-term culture. Compared to MD-astrocytes, these immunopanned astrocytes, which we refer to in this paper as IP-astrocytes, maintain gene profiles in culture that much more closely mimic their acutely purified state. Lastly using this new IP-astrocytes preparation, we begin to unravel some of the fundamental functional properties of astrocytes.

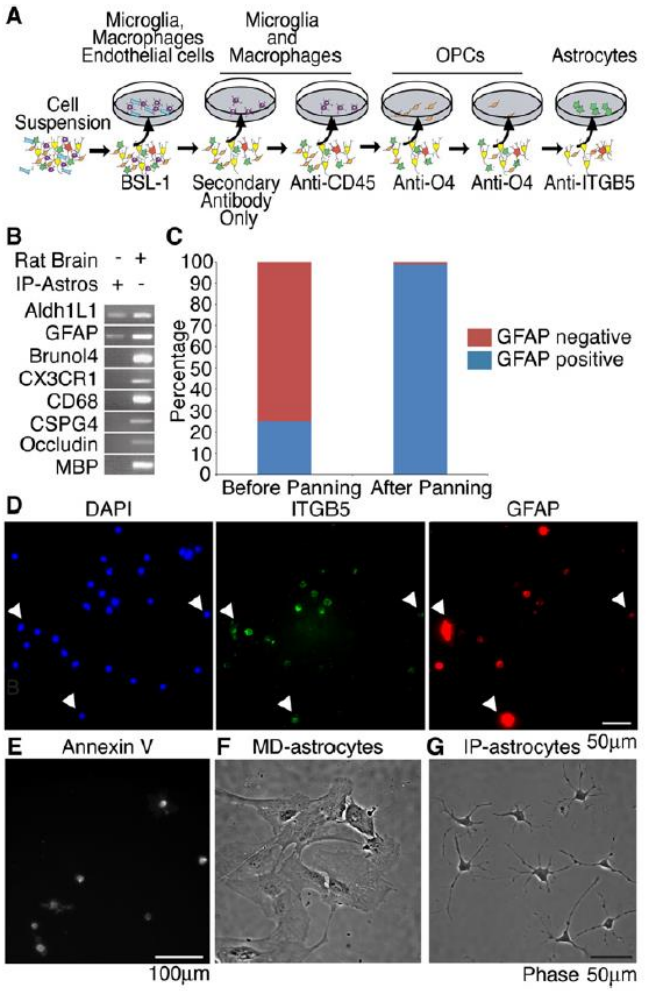


Figure 1. Establishment of an Immunopanning Protocol for Rat Astrocytes

(A) Cortical suspensions were passed over successive panning plates to remove endothelial cells and microglia (BSL-1), microglia and macrophages (secondary only plate), microglia (CD45), oligodendrocyte precursor cells (O4), and finally a positive panning plate for astrocytes (ITGB5).

(B) Purity of IP-astrocytes validated by RT-PCR and (C) immunostaining with GFAP. Before immunopanning, the whole cell brain suspension contained 25.1% $GFAP^+$ cells, after immunopanning, the isolated cells were 98.7% $GFAP^+$.

(D) ITGB5 was present on all astrocytes. All cells in cortical cell suspensions that were $GFAP^+$ were also $ITGB5^+$ (arrowheads).

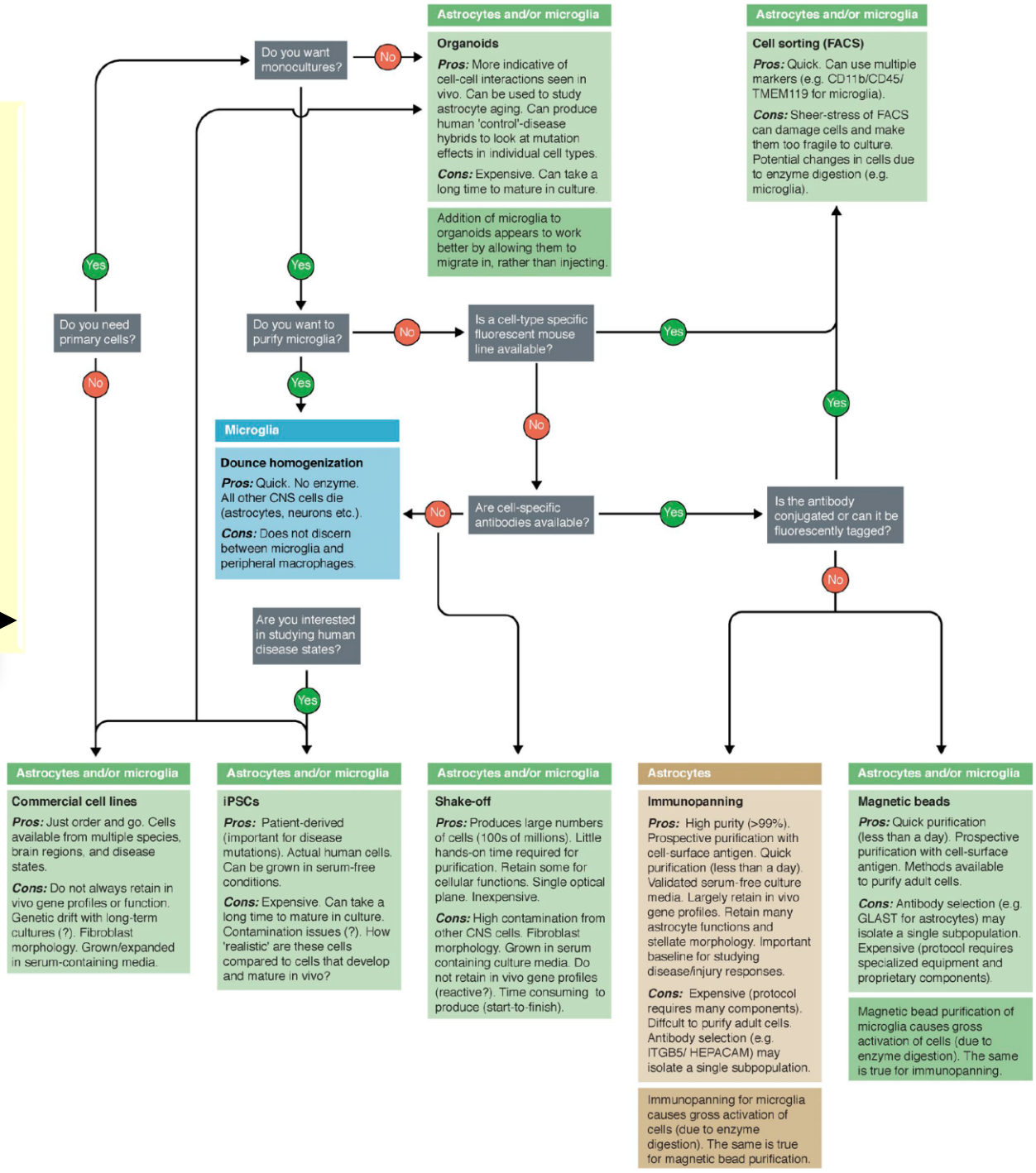
(E) Astrocytes are positive for the apoptotic marker Annexin V.

(F) MD-astrocytes had flat and fibroblast-like morphologies at 14DIV.

(G) IP-astrocytes cultured with HBEGF for 14DIV were healthy and extended multiple processes.

What is the best method for astrocyte purification?

Purification flow chart of astrocytes and microglia



Guttenplan & Liddelow, 2018
J. Experimental Medicine
http://doi.org/10.1084/jem.20180200

Table 1. **Common astrocyte markers and reagents**

Gene (protein)	Labeled cells (CNS)	Genetic lines	Antibodies	Notes	Reference
Gfap (GFAP)	Astrocytes + NPCs	Fluorescent reporter, Cre, CreERT2	Y	Upregulated in some reactive astrocytes	Brenner et al., 1994; Zhuo et al., 2001; Su et al., 2004; Ganat et al., 2006; Liu et al., 2010
Aldh1l1 (ALDH1L1)	Astrocytes	Fluorescent reporter, CreERT2 (new)	Y		Cahoy et al., 2008; Srinivasan et al., 2016; Winchenbach et al., 2016
Slc1a3 (GLAST)	Astrocytes + NPCs	Fluorescent reporter, CreERT	Y	Developed by Jeremy Nathans (Mouse Genome Informatics)	Regan et al., 2007; Kang et al., 2010; de Melo et al., 2012; Wang et al., 2012
Slc1a2 (GLT1)	Astrocytes + NPCs	Fluorescent reporter	Y		Regan et al., 2007; Yang et al., 2011
S100b (S100B)	Astrocytes + OL lineage	Fluorescent reporter, CreERT2	Y		Zuo et al., 2004; McMahon et al., 2008; Harding et al., 2011
Gjb6 (CX30)	Astrocytes + NPCs	CreERT2	Y		Slezak et al., 2007; Srinivasan et al., 2016
Slc6a11 (GAT3)	Astrocytes + NPCs	CreERT2	Y		Srinivasan et al., 2016
Nes (NESTIN)	Astrocytes + NPCs	Fluorescent reporter, Cre, CreERT2	Y	Upregulated in some reactive astrocytes	Betz et al., 1996; Tronche et al., 1999; Battiste et al., 2007; Lagace et al., 2007
Vim (VIMENTIN)	Astrocytes + NPCs	Fluorescent reporter, LacZ	Y	Upregulated in some reactive astrocytes	Colucci-Guyon et al., 1994
C3	Astrocyte + certain Cx3cr1^{+} cells	Fluorescent reporter (not finalized)	Y (human)	Upregulated in A1 reactive astrocytes; in situ hybridization required for murine tissue	Liddelow et al., 2017

OL, oligodendrocyte; Y, yes.

Guttenplan & Liddelow, 2018
J. Experimental Medicine
http://doi.org/10.1084/jem.20180200

SCIENTIFIC REPORTS
nature research

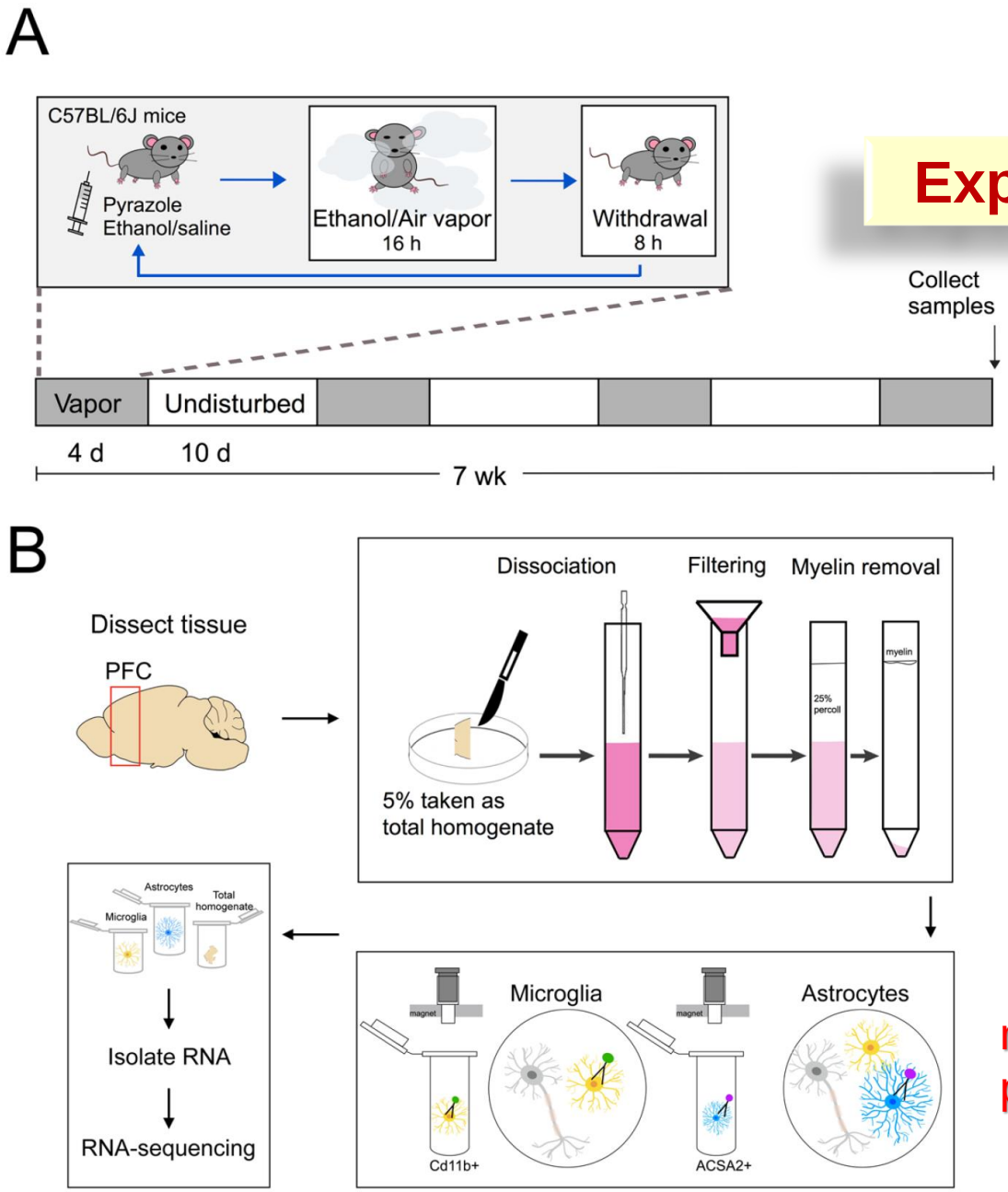
Glial gene networks associated with alcohol dependence

Emma K. Erickson, Yuri A. Blednov, R. Adron Harris & R. Dayne Mayfield

Chronic alcohol abuse alters the molecular structure and function of brain cells. Recent work suggests adaptations made by glial cells, such as astrocytes and microglia, regulate physiological and behavioral changes associated with addiction. Defining how alcohol dependence alters the transcriptome of different cell types is critical for developing the mechanistic hypotheses necessary for a nuanced understanding of cellular signaling in the alcohol-dependent brain. We performed RNA-sequencing on total homogenate and glial cell populations isolated from mouse prefrontal cortex (PFC) following chronic intermittent ethanol vapor exposure (CIE). Compared with total homogenate, we observed unique and robust gene expression changes in astrocytes and microglia in response to CIE. Gene co-expression network analysis revealed biological pathways and hub genes associated with CIE in astrocytes and microglia that may regulate alcohol-dependent phenotypes. Astrocyte identity and synaptic calcium signaling genes were enriched in alcohol-associated astrocyte networks, while TGF-β signaling and inflammatory response genes were disrupted by CIE treatment in microglia gene networks. Genes related to innate immune signaling, specifically interferon pathways, were consistently up-regulated across CIE-exposed astrocytes, microglia, and total homogenate PFC tissue. This study illuminates the cell-specific effects of chronic alcohol exposure and provides novel molecular targets for studying alcohol dependence.

Erikson et al, 2019
Scientific Reports
https://doi.org/10.1038/s41598-019-47454-4

Experimental design



magnetic bead
purification tecnnique

Figure 1. Schematic of alcohol exposure and cell isolation methods. (**A**) Chronic intermittent ethanol vapor procedure. (**B**) Cell type isolation procedure.

Results

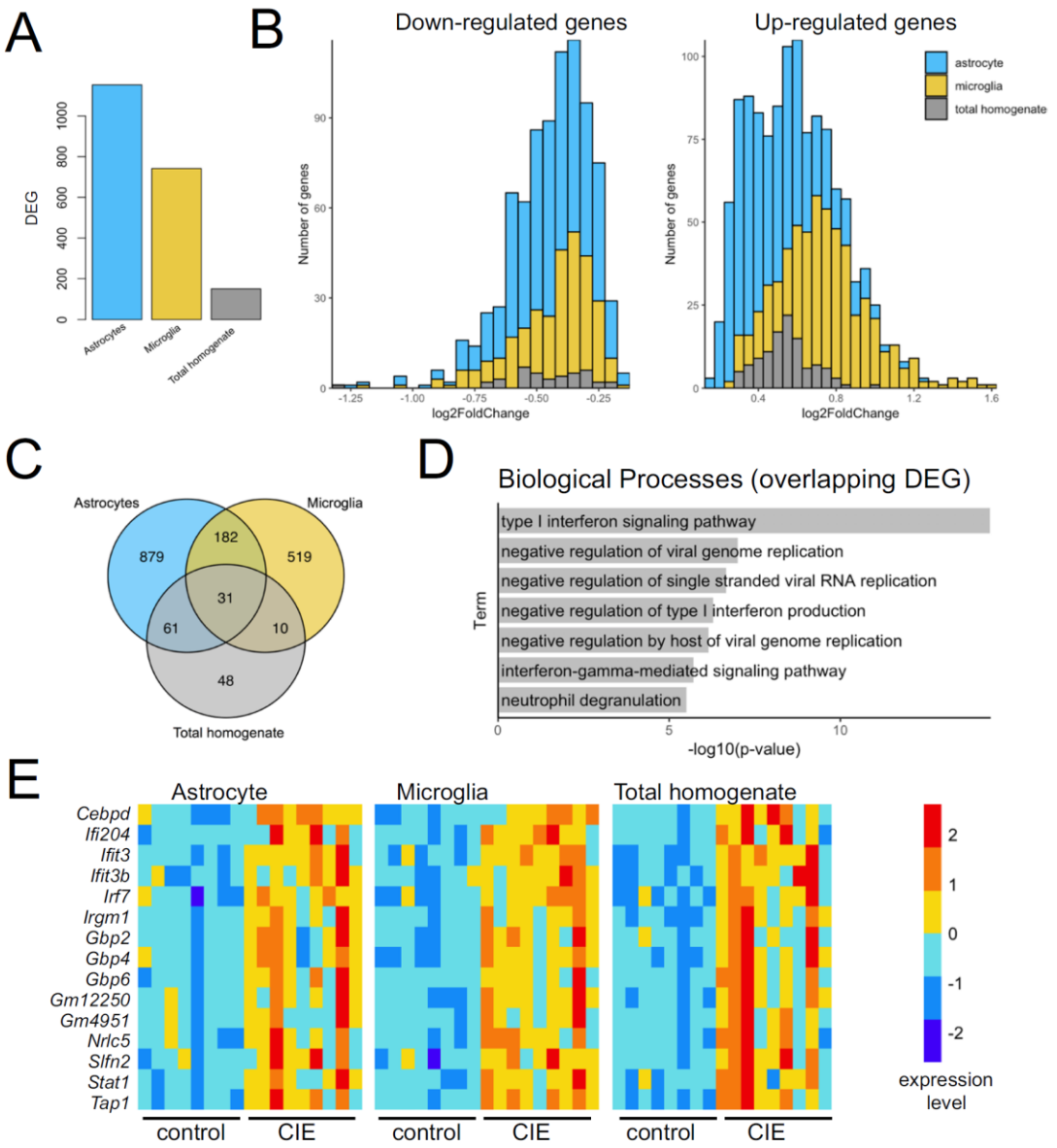


Figure 2. Differential expression comparison by cell type. (**A**) Number of CIE-induced differentially expressed genes (DEG) ($p_{adj} < 0.05$) identified in astrocytes, microglia, and total homogenate. (**B**) Histograms plotting magnitude of fold changes for up- and down-regulated DEG identified in each tissue type. (**C**) Overlap of DEG between astrocytes, microglia, and total homogenate. (**D**) Biological process enrichment results of overlapping DEG across all cell types. (**E**) Heat maps displaying expression levels of interferon-related DEG across control and CIE-treated samples for astrocytes, microglia, and total homogenate. Color indicates expression level (regularized log transformed gene counts).

Erikson et al, 2019
Scientific Reports
https://doi.org/10.1038/s41598-019-47454-4

Validation of RNA-seq results by Immunocytochemistry

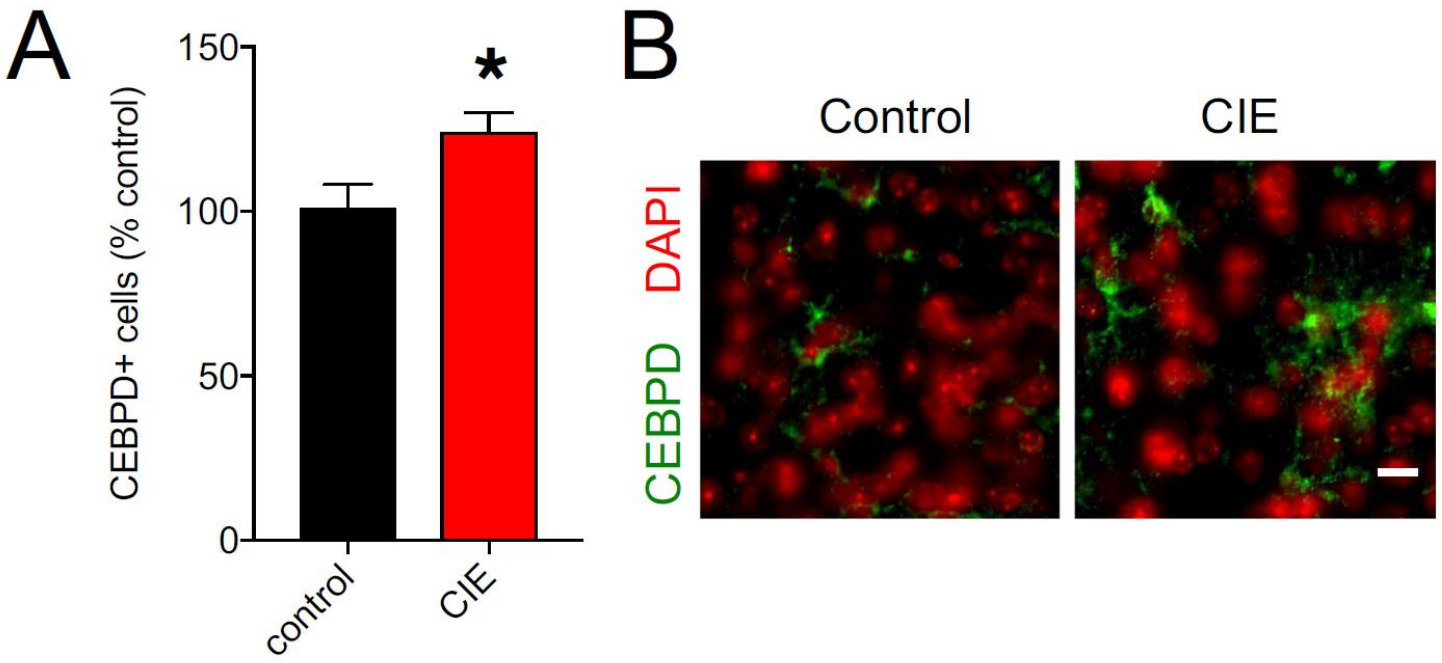


Figure 6. Immunostaining of CEBPD in PFC tissue from control or CIE-treated mice. (**A**) Quantification of CEBPD+ cells. (**B**) Representative images of CEBPD staining in PFC. Scale bar = 20 μm.

Erikson et al, 2019
Scientific Reports
https://doi.org/10.1038/s41598-019-47454-4

Methods to study astrocyte-neuron interactions in vitro

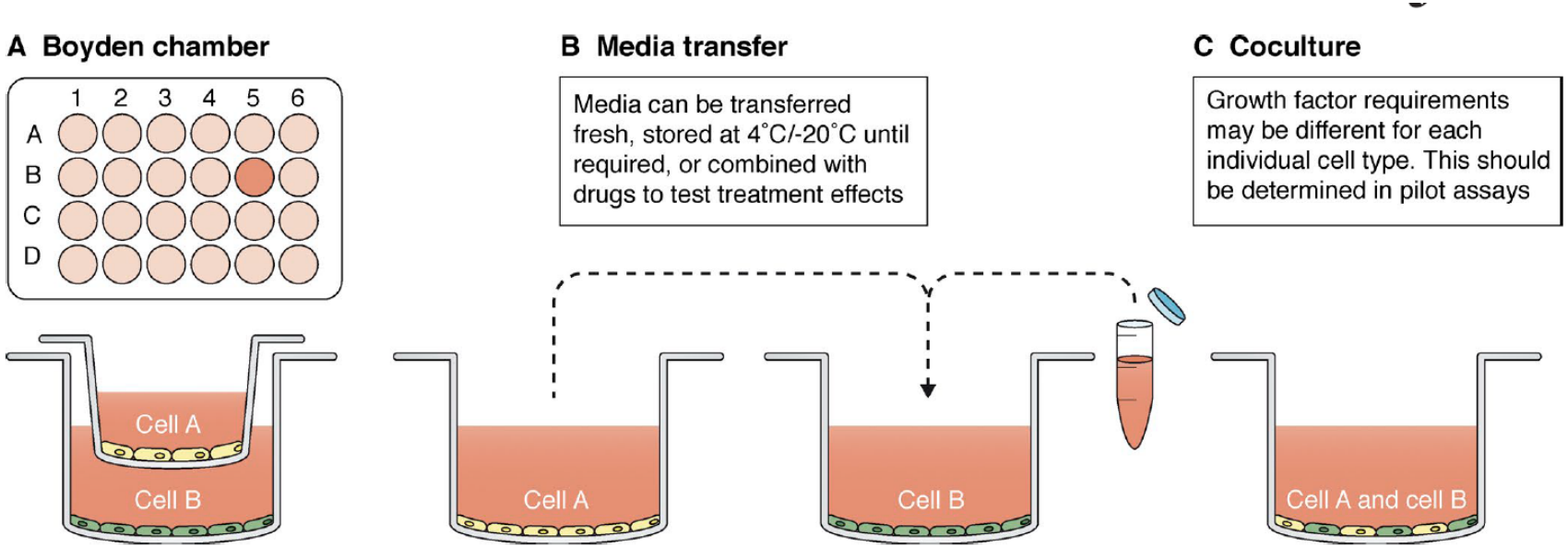


Figure 2. **Cell–cell interactions in a culture dish.** Several methods available for investigating interactions between cell types. **(A)** Boyden chamber: Two cell types grown in the same well but separated via semipermeable membrane. This retains bidirectional cell–cell communication via secreted cues. **(B)** Media transfer: Individual cells grown in isolation with exchanged media containing secreted factors. Benefits include ability to produce and store conditioned medium in bulk (if factors are stable at storage temperatures) and amenity to neutralizing antibodies or drugs. **(C)** Coculture experiments: Two (or more) types of cells in the same culture well, allowing for communication by secreted factors and direct cell–cell contact.

Guttenplan & Liddelow, 2018
J. Experimental Medicine
http://doi.org/10.1084/jem.20180200

Table 2. **Common transcriptome resources**

Website	Laboratory	Reference	Focus
http://igc1.salk.edu:3838/astrocyte_aging_transcriptome/	Allen	Boisvert et al., 2018	Aging mouse astrocytes, multiple brain areas
http://www.brainrnaseq.org/	Barres	Zhang et al., 2014, 2016; Bennett et al., 2016; Clarke et al., 2018	Glial cell specific in mouse and human; mouse microglia throughout development; aging mouse astrocytes, multiple brain areas
http://bioinf.nl:8080/GOAD2/	Boddeke	Holtman et al., 2015	Repository of multiple other published glia sequencing datasets
http://shiny.maths.usyd.edu.au/Ellis/MicrogliaPlots	Bradshaw		Aged human microglia
http://astrocyternaseq.org/	Khakh	Srinivasan et al., 2016; Chai et al., 2017	Adult mouse brain regional differences in astrocytes
http://www.mousebrain.org/	Linnarsson	Zeisel et al., 2018	Single-cell analysis of many cell types from different brain regions and developmental stages of the mouse
http://www.dropviz.org/	McCarroll	Saunders et al., 2018	Single-cell analysis of many cell types from different mouse brain regions
https://astrocyte.rnaseq.sofroniewlab.neurobio.ucla.edu/	Sofroniew	Anderson et al., 2016	Mouse astrocyte reactivity in spinal cord injury and inflammation
http://www.microgliasinglecell.com/	Stevens/ McCarroll	Hammond et al., 2018	Single cell microglia during age, by sex, and in demyelinating disease model

Additional datasets for non-glial CNS cells are reviewed in Keil et al., 2018.

Guttenplan & Liddelow, 2018
J. Experimental Medicine
http://doi.org/10.1084/jem.20180200

Astrocyte heterogeneity

Farmer & Murai, 2017
Frontiers in Cellular Neuroscience
DOI 10.1016/j.cell.2009.09.025

FIGURE 1 | Continued
PF-PC synapses activates calcium permeable ionotropic AMPA receptors and causes and influx of Ca^{2+} into astrocytes. PC, Purkinje cell; BG, Bergmann glia; ML, molecular layer; PCL, Purkinje cell layer; GCL, granule cell layer; PF, parallel fiber. **(C)** Astrocytes in arcuate nucleus of the hypothalamus are critical regulators of satiety and energy homeostasis. They respond directly to the hormones insulin, leptin and ghrelin by modulating glucose transport and the synaptic activity of hypothalamic neurons. Ultimately, astrocytic metabolic hormone signaling is necessary for the homeostatic maintenance of blood glucose levels and the regulation of feeding behavior. **(D)** Astrocytes of the dorsal suprachiasmatic nucleus (dSCN) are critical players in the maintenance of circadian rhythm. (**D**, upper panel) The levels of calcium signaling neurons and astrocytes are anti-phase; neurons are active during the day while astrocytes are active during the night. (**D**, lower panels) Simulated plots of periodic wheel running activity of mice under multiple conditions. The motor activity of mice is largely constrained to dark periods during equal length light and dark cycles. When kept in constant darkness, circadian period is only slightly shifted as revealed by their motor activity. However, when the intrinsic circadian clock of dSCN neurons is lengthened, the mice display a large shift in the periods of motor activity. Interestingly, when the same manipulation to lengthen the intrinsic circadian period is performed on dSCN astrocytes, the mice show a shift in behavior that resembles the neuronal manipulation.

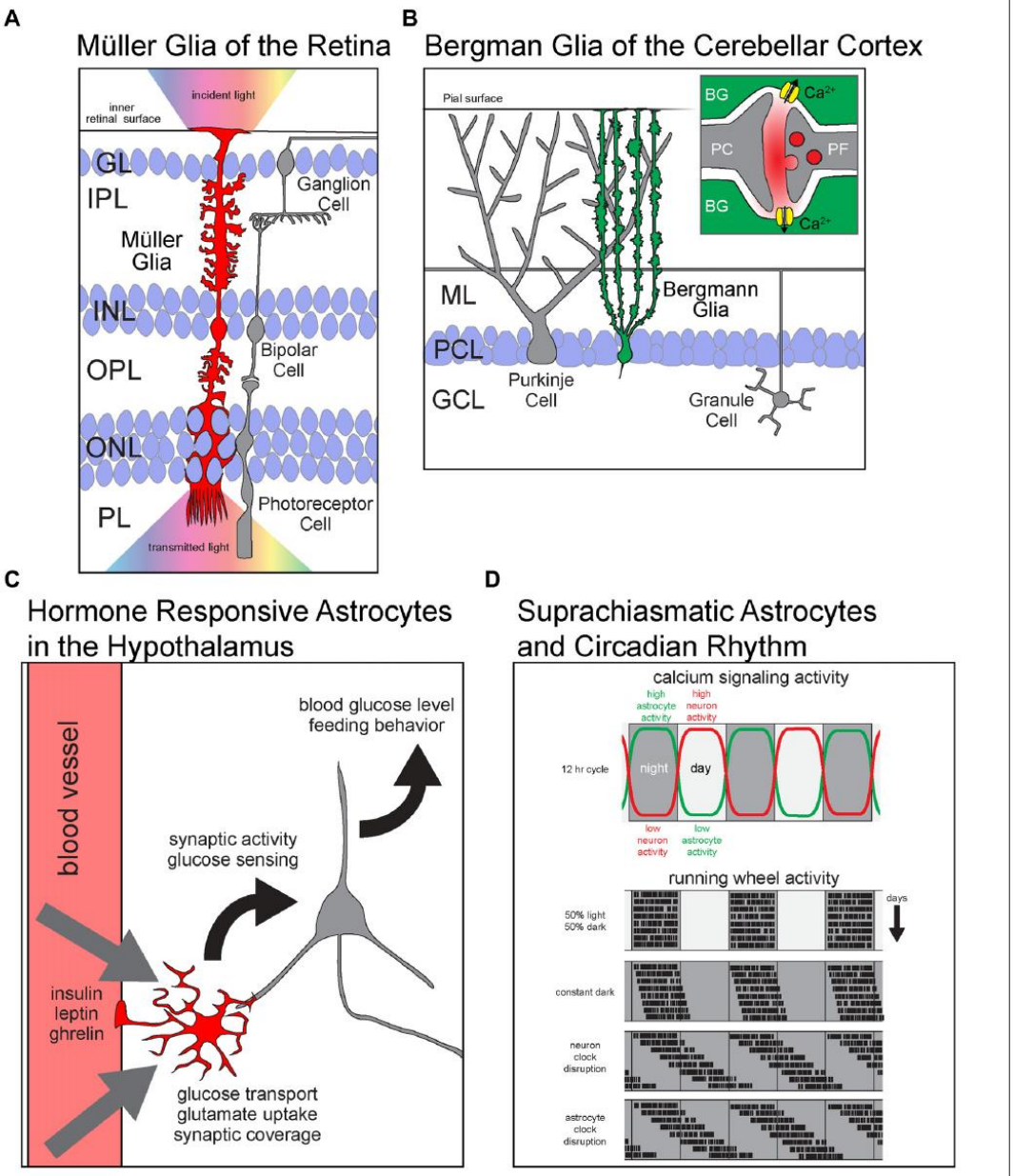


FIGURE 1 | Astrocyte diversity across brain regions. Schematics showing region specific properties and functions of different astrocyte types. **(A)** Müller glia (MG) of the retina are radially polarized astrocytes that span all retinal layers. With endfeet attached to the inner retinal surface and soma in the inner nuclear layer (INL), they elaborate processes into every layer of the retina. MG facilitate the transmission of light (rainbow) from the inside of the eye (up) across the retina to the photoreceptors in PL. GL, granule layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PL, photoreceptor layer. **(B)** BG are radially polarized astrocytes that manage the glutamatergic synapse-rich neuropil of the cerebellar cortex. (**B**, inset) Glutamate released at

(*Continued*)

Astrocyte heterogeneity in neuropathology

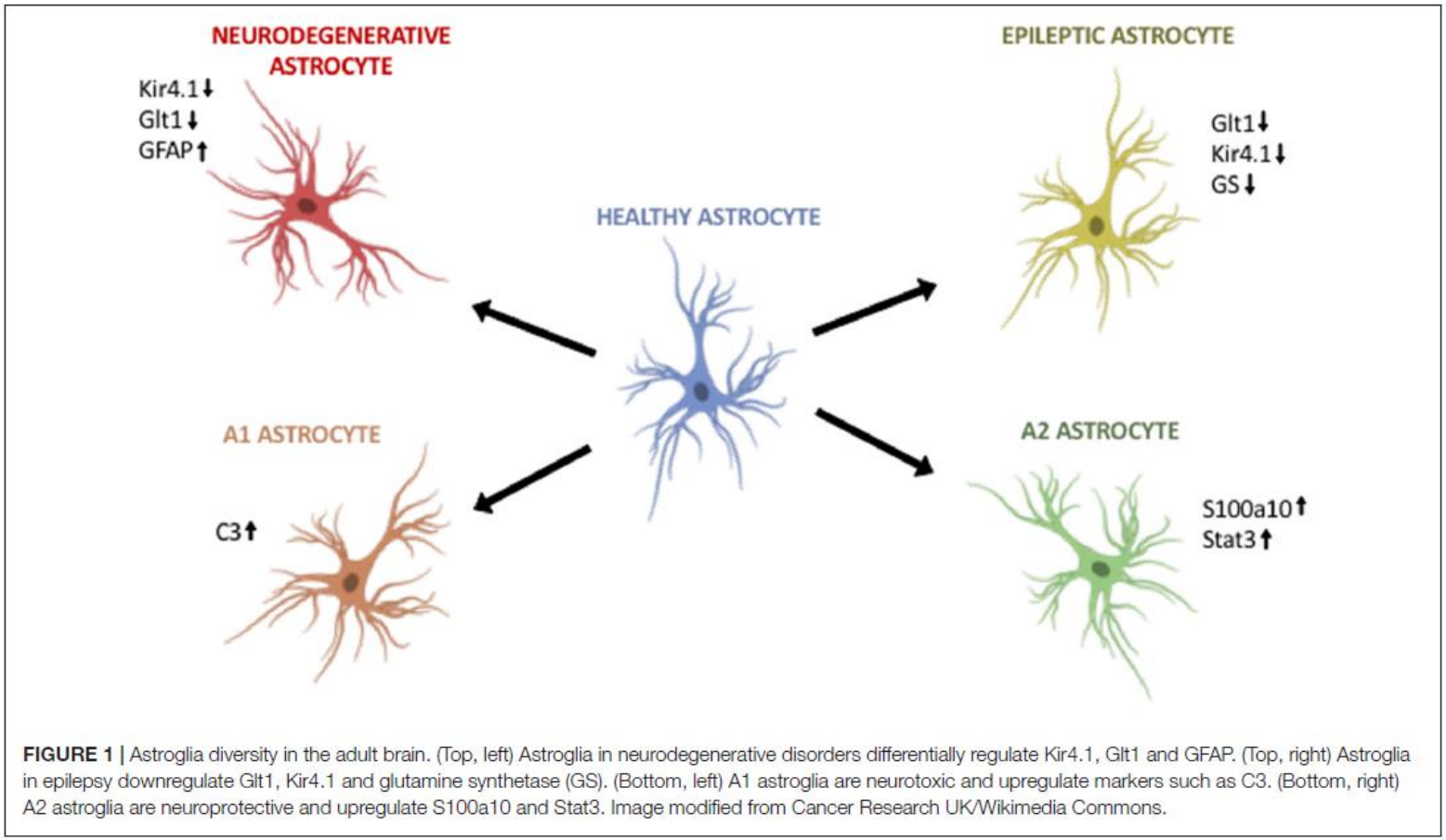
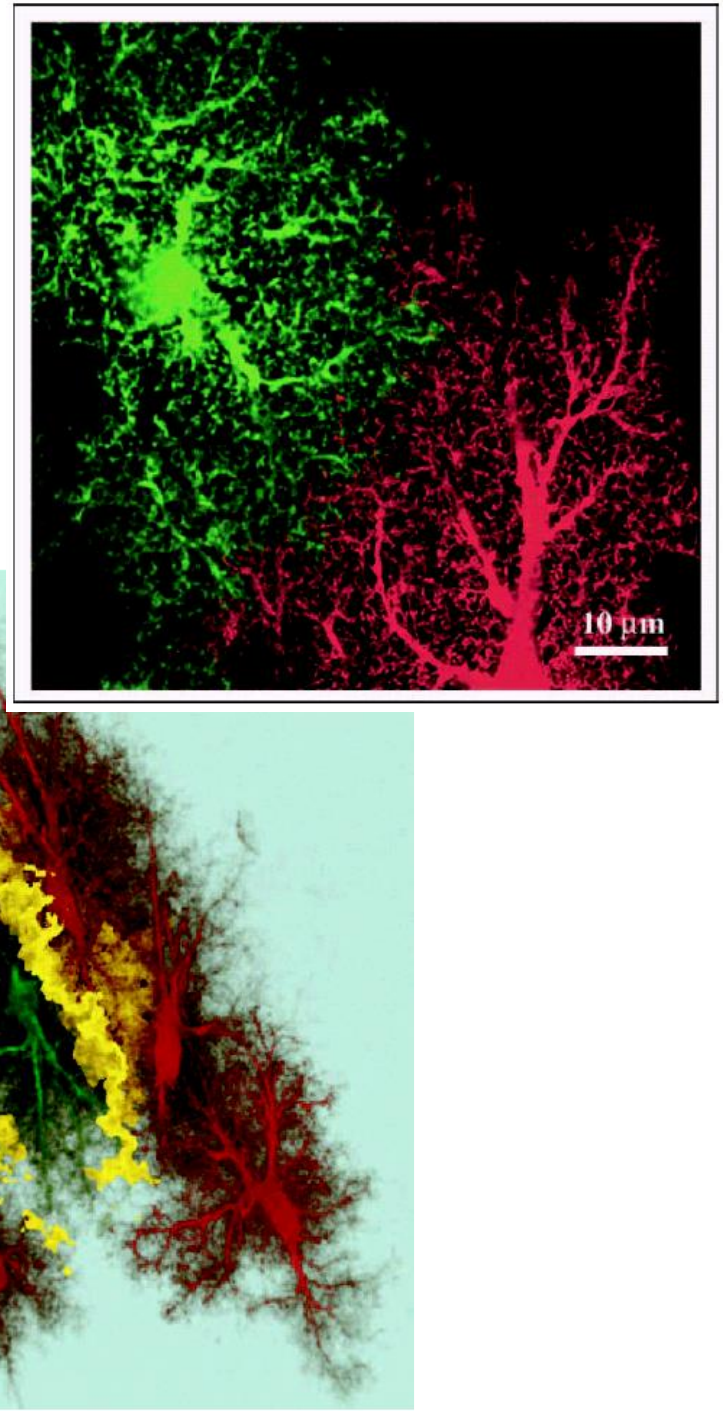


FIGURE 1 | Astroglia diversity in the adult brain. (Top, left) Astroglia in neurodegenerative disorders differentially regulate Kir4.1, Glt1 and GFAP. (Top, right) Astroglia in epilepsy downregulate Glt1, Kir4.1 and glutamine synthetase (GS). (Bottom, left) A1 astroglia are neurotoxic and upregulate markers such as C3. (Bottom, right) A2 astroglia are neuroprotective and upregulate S100a10 and Stat3. Image modified from Cancer Research UK/Wikimedia Commons.

Miller, 2018
Frontiers in Cellular Neuroscience
doi: 10.3389/fncel.2018.00401

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

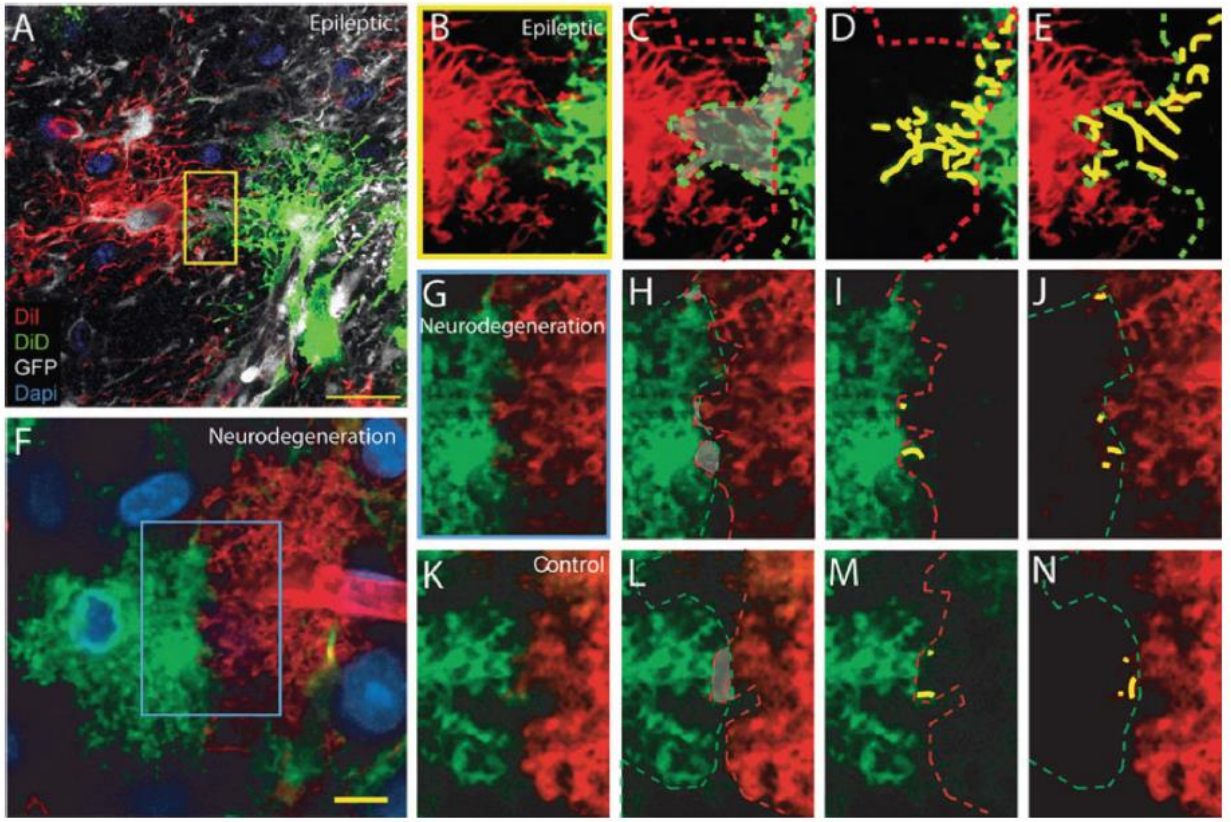
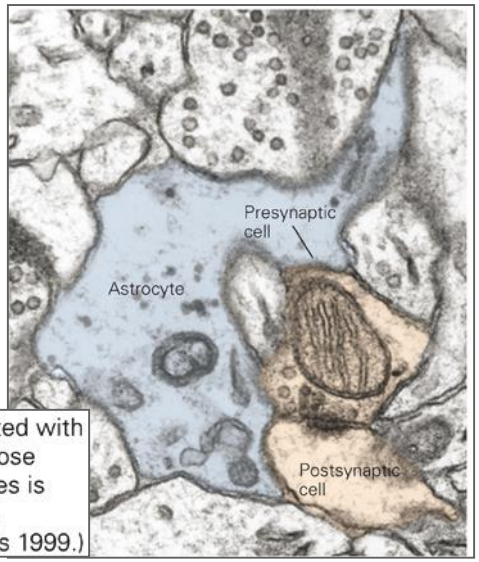


Fig. 2.
Astrocytic domain organization varies with pathology. The domain organization of protoplasmic astrocytes is lost in epileptic brains, but maintained in neurodegeneration. (**a**) Reactive astrocytes 1 week post-iron injection lose the domain organization. Diolistic labelling of the cortex of 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 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.)

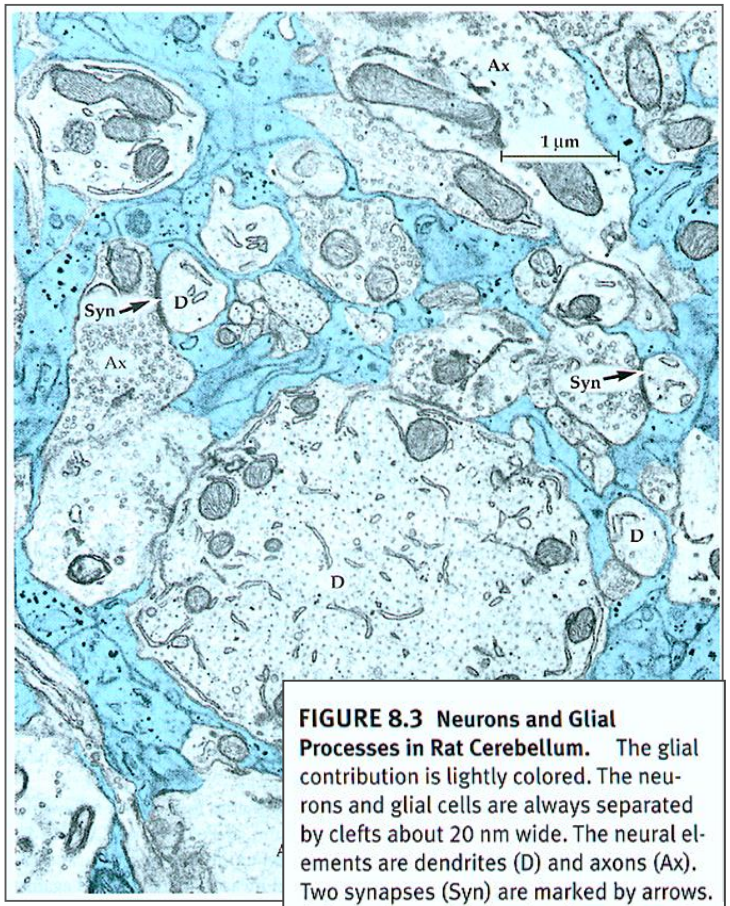
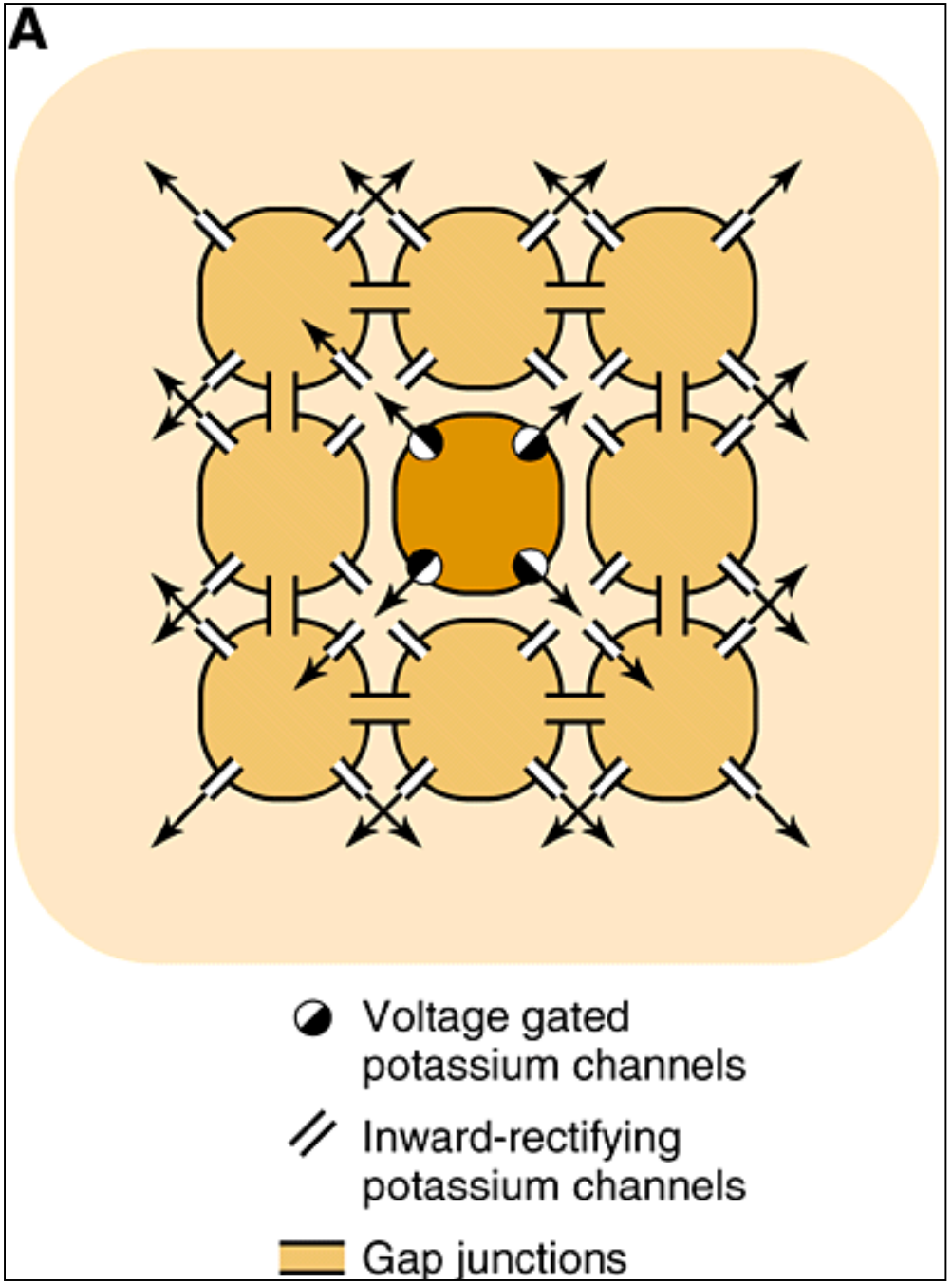


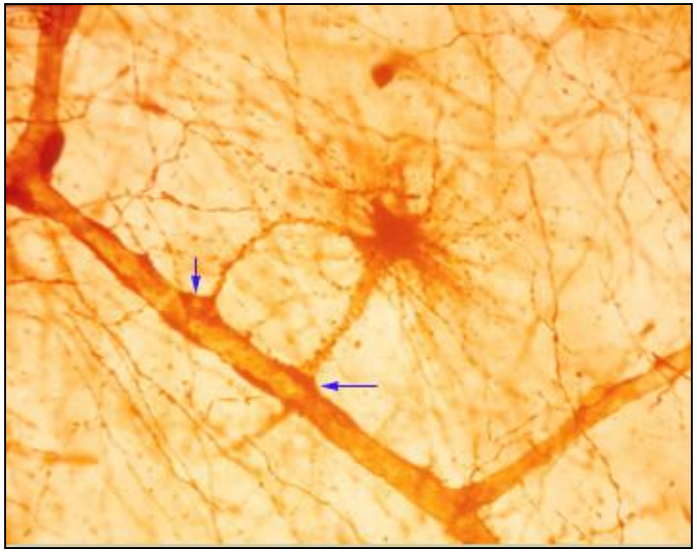
FIGURE 8.3 Neurons and Glial Processes in Rat Cerebellum. The glial contribution is lightly colored. The neurons and glial cells are always separated by clefts about 20 nm wide. The neural elements are dendrites (D) and axons (Ax). Two synapses (Syn) are marked by arrows. (After Peters, Palay, and Webster,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

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.



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.

Astrocytes and brain metabolism – glucose and glutamate pathways

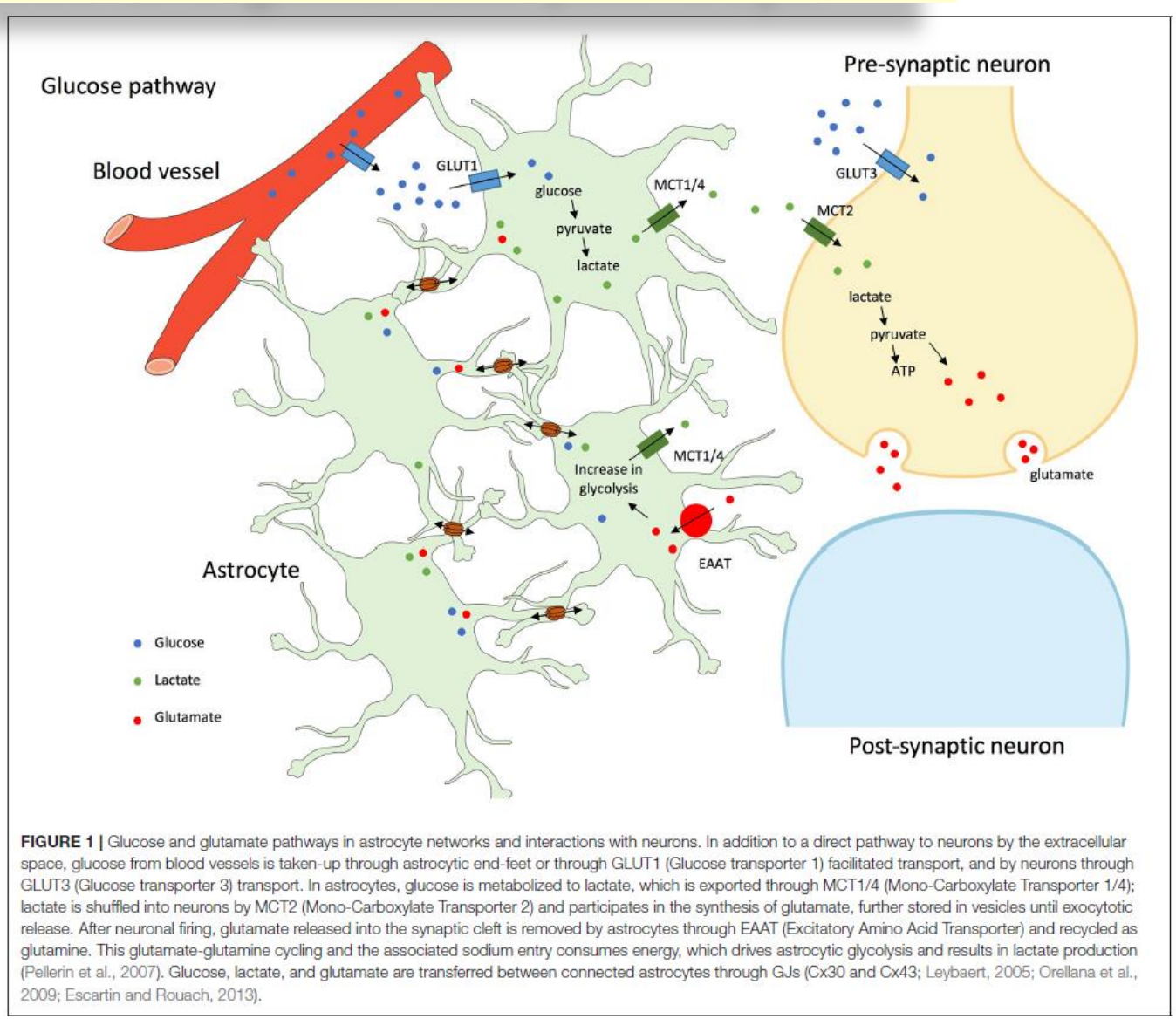


FIGURE 1 | Glucose and glutamate pathways in astrocyte networks and interactions with neurons. In addition to a direct pathway to neurons by the extracellular space, glucose from blood vessels is taken-up through astrocytic end-feet or through GLUT1 (Glucose transporter 1) facilitated transport, and by neurons through GLUT3 (Glucose transporter 3) transport. In astrocytes, glucose is metabolized to lactate, which is exported through MCT1/4 (Mono-Carboxylate Transporter 1/4); lactate is shuffled into neurons by MCT2 (Mono-Carboxylate Transporter 2) and participates in the synthesis of glutamate, further stored in vesicles until exocytotic release. After neuronal firing, glutamate released into the synaptic cleft is removed by astrocytes through EAAT (Excitatory Amino Acid Transporter) and recycled as glutamine. This glutamate-glutamine cycling and the associated sodium entry consumes energy, which drives astrocytic glycolysis and results in lactate production (Pellerin et al., 2007). Glucose, lactate, and glutamate are transferred between connected astrocytes through GJs (Cx30 and Cx43; Leybaert, 2005; Orellana et al., 2009; Escartin and Rouach, 2013).

Charveriat et al., 2017
Frontiers in Cellular Neuroscience
doi: 10.3389/fncel.2017.00174

Elimination of Glu and GABA from the intersynaptic space by astrocytes

Glu = glutamate

GS = glutamine sintethetase

Gln = glutamine

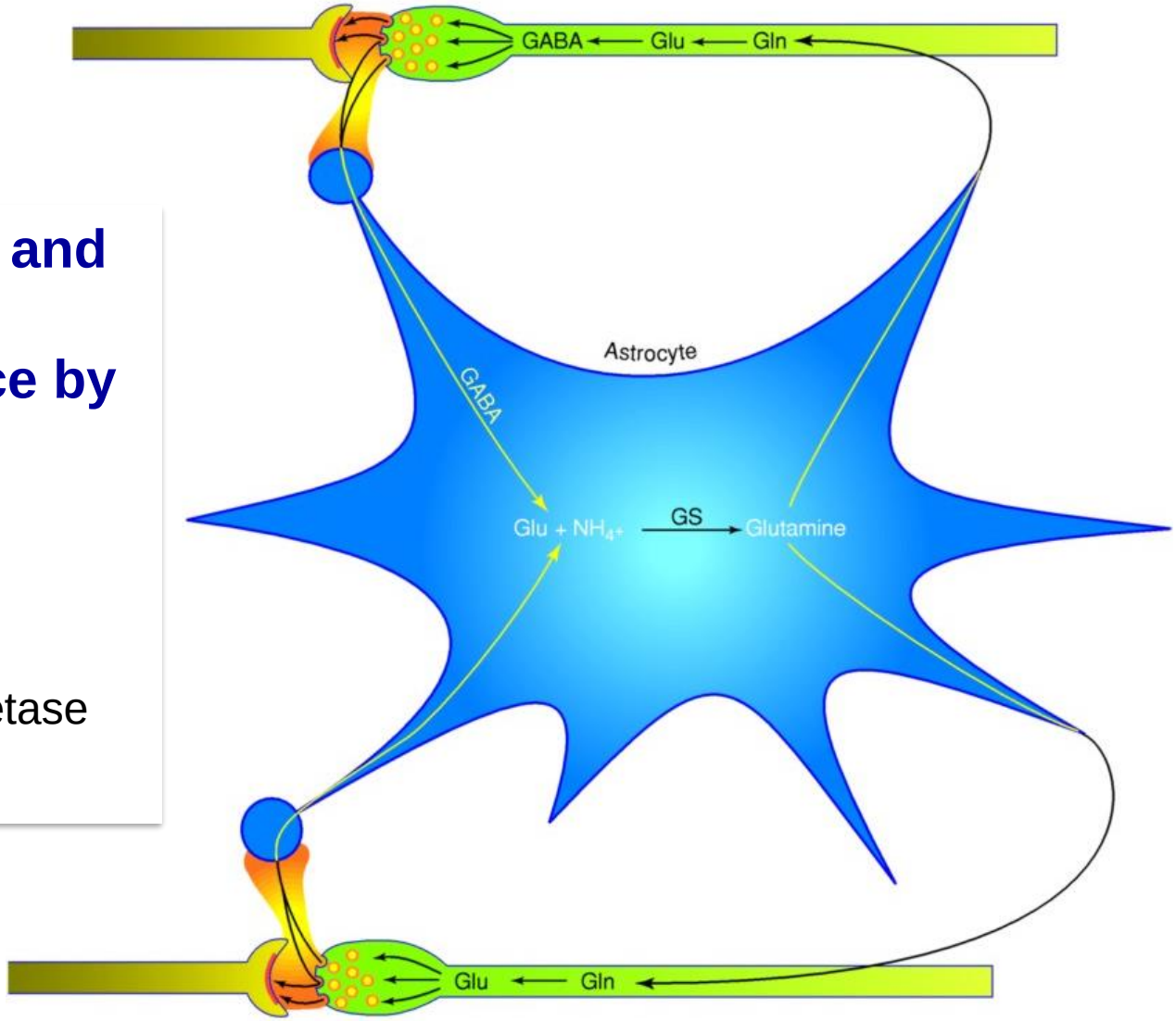
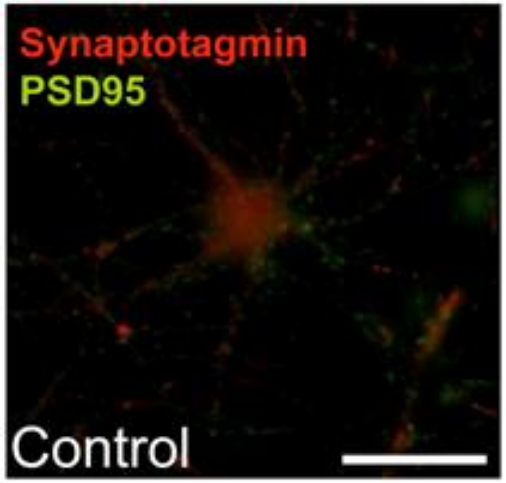
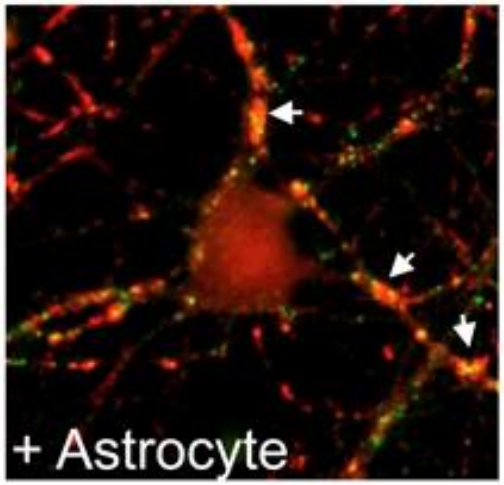


FIGURE 19 The glutamate–glutamine cycle is an example of a complex mechanism that involves an active coupling of neurotransmitter metabolism between neurons and astrocytes. The systems of exchange of glutamine, glutamate, GABA, and ammonia between neurons and astrocytes are highly integrated. The postulated 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.

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

Dynamic association of astrocytic processes with developing and mature synapses

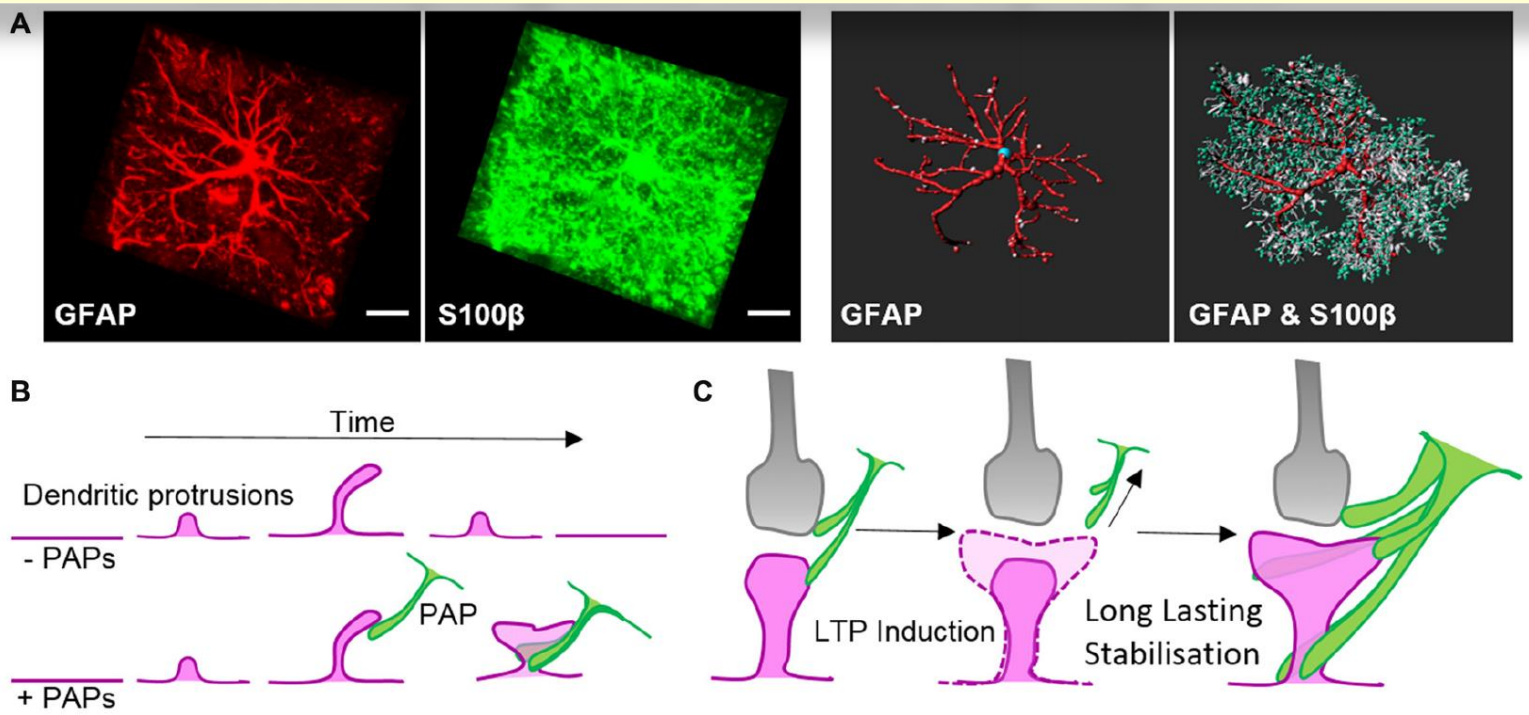
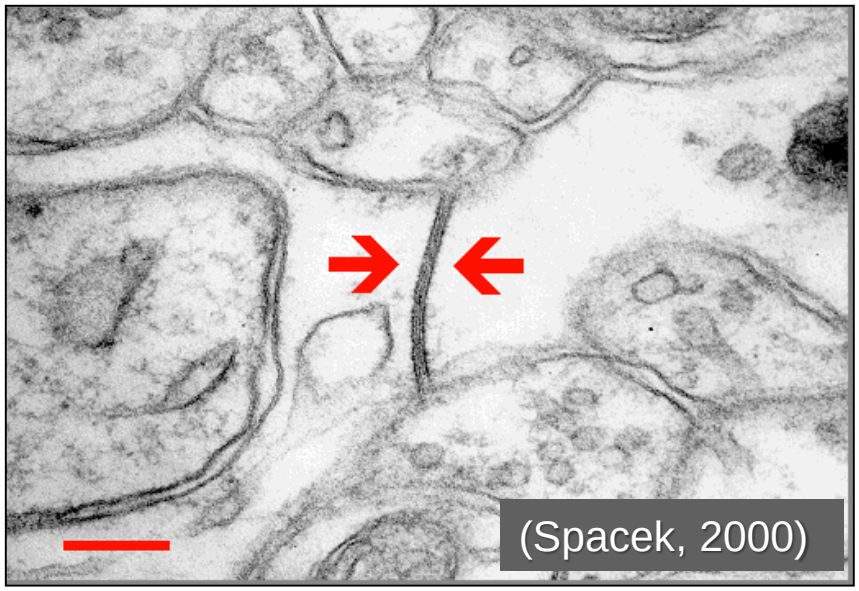


FIGURE 2 | Morphology of astrocytes *in vivo* and their dynamic association with synapses. **(A)** *Z*-projection of a cortical rat astrocyte (P14), stained for glial fibrillary acidic protein (GFAP; red) and S100β (green), by confocal microscopy after tissue clearance (left). Scale bars: 10 μm. 3D rendering and morphometric analyses show the restriction of the intermediate filament protein GFAP to main processes, which are decorated with myriads of fine S100β-positive processes (right; with permission from Murk et al., 2013). **(B)** Schematic of dendritic filopodia, with the precursors of dendritic spines, emerging from dendrites (magenta) in the absence (top) or presence of perisynaptic astrocytic processes (PAPs; green, bottom). Without the support of astrocytic processes, sprouting dendritic filopodia have a short lifespan and are likely to retract. Astrocytic processes contact filopodia-like protrusions, which then exhibit an increased stability and higher tendency to develop into mature dendritic spines. **(C)** Schematic of a mature tripartite synapse consisting of the pre-synapse (gray), post-synapse (magenta) and PAPs (green), which respond to long-term potentiation (LTP) with structural changes. Induction of LTP transiently enhances the motility and retraction of PAPs allowing growth of the postsynaptic dendritic spine to occur. Subsequent to dendritic spine remodeling, PAPs intensify their coverage of synapses.

PAPs = perisynaptic astrocytic processes

Schiweck et al., 2018
Frontiers in Cellular Neuroscience
doi: 10.3389/fncel.2018.00261

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 progression

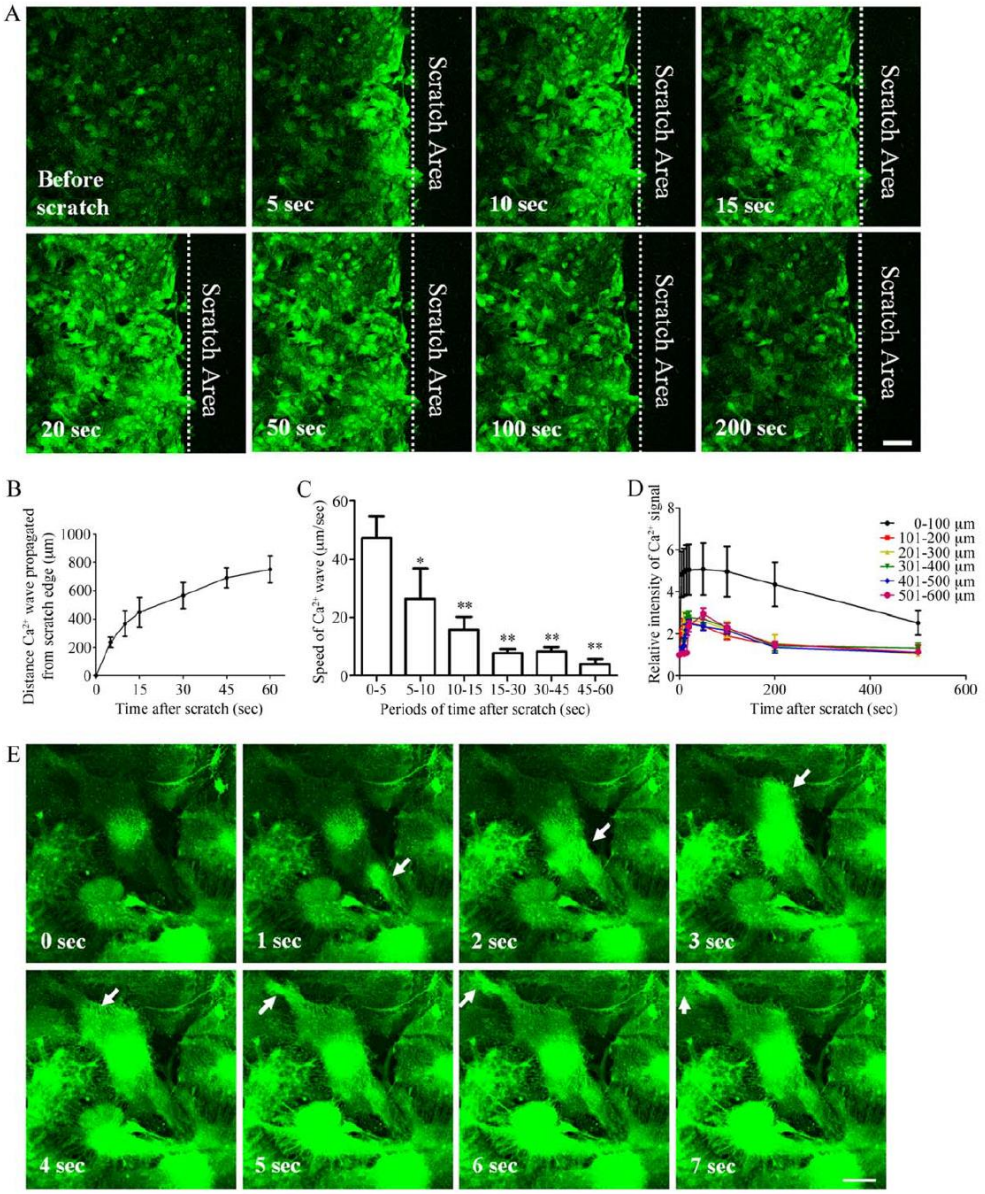
Stimulation of one astrocyte can cause a calcium response in a subset of neighboring astrocytes, but not others, suggesting distinct networks of astrocytes.

DOI: 10.1002/glia.22577

An in vitro scratch injury model to study spreading of calcium waves in astrocytes

- Make a scratch in primary cultures of cerebral cortical astrocytes
- This scratch induces an influx of calcium in the form of waves spreading away from the wound through gap junctions

- Using the calcium blocker BAPTA-AM and the JNK inhibitor SP600125, Authors demonstrate that the calcium wave triggered the activation of JNK, which then phosphorylated the transcription factor c-Jun to facilitate binding of AP-1 to the GFAP gene promoter to switch on GFAP upregulation.



Watch movies at:
https://onlinelibrary.wiley.com/doi/full/10.1002/glia.22577

FIGURE 1: Scratch-induced calcium waves in primary cultures of astrocytes. (**A**) Time lapse recording of a scratch induced calcium wave between astrocytes with the calcium indicator Fluo-3 AM. The time labels reflect the time points after scratch (s). Dotted lines show the scratch edge. Bar = 100 μm. (**B**) Distance of calcium wave propagation from the scratch edge at different time points after scratch. n = 3. (**C**) Speed of calcium wave propagation after scratch. *P<0.05, **P<0.01 compared with 0–5 s, n = 3. (**D**) Change of calcium signal at different distances from the scratch edge (n = 5). (**E**) Time lapse recording of a scratch-induced calcium wave in single astrocytes with the calcium indicator Fluo-3 AM. The time labels reflect the time points (s) after the calcium signal was transmitted into the cell in the center of the figures. Arrows show the edge of calcium wave propagation. Bar = 20 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Mechanisms of Ca++ signaling in astrocytes

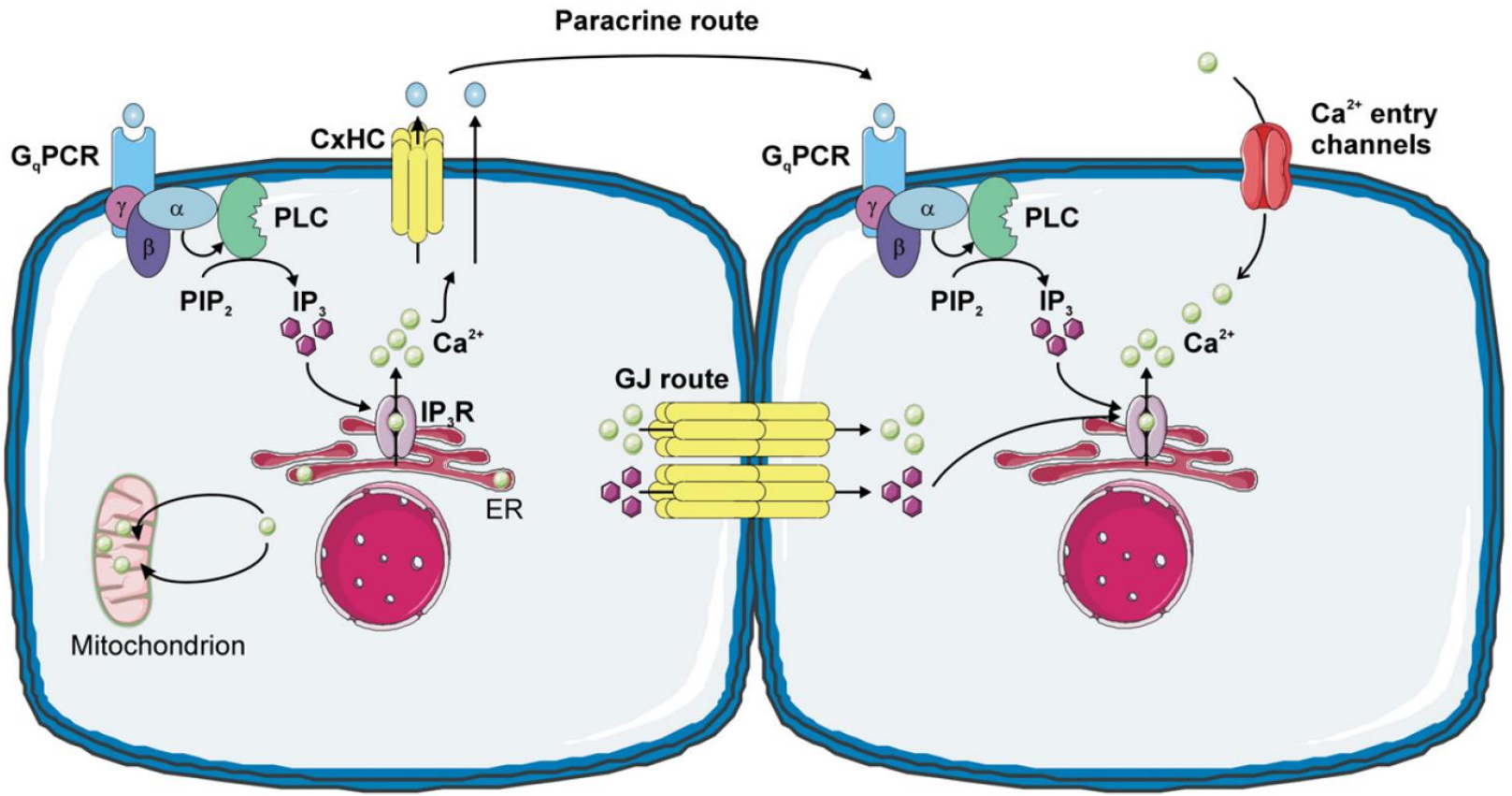
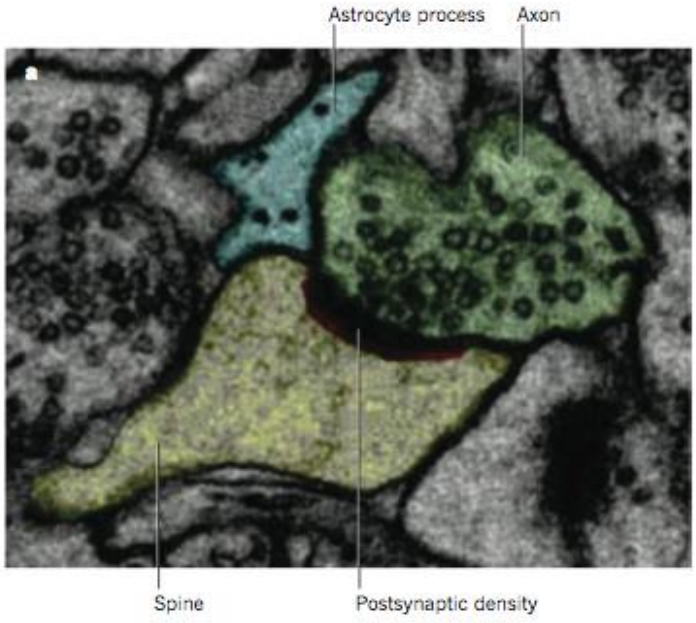


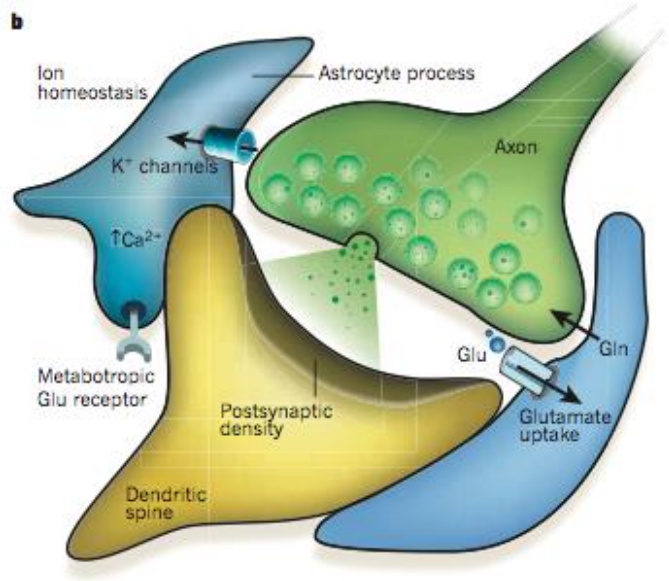
Fig. 2. Mechanisms underlying intercellular Ca^{2+} signaling. The activation of plasma membrane GPCRs results in the PLC-mediated synthesis of IP_3 and subsequent IICR from the ER. Mitochondrial Ca^{2+} uptake prevents Ca^{2+} from reaching deleterious levels in the cytoplasm. The intracellular Ca^{2+} signal can propagate to neighboring cells via two routes, a direct one involving GJs, with IP_3 as the primary coordinating messenger, and an indirect one that is mediated by the release of paracrine messengers. Here, ATP and glutamate are the prototypical paracrine molecules released into the extracellular environment via multiple mechanisms including HCs, a vesicular pathway or channels such as the $P2X_7$ receptor. They diffuse in the extracellular space and activate their corresponding GPCRs or channels on neighboring cells. Regenerative mechanisms include the Ca^{2+}-dependent activation of PLCδ or stimulation of paracrine release mechanisms. (CxHC, connexin hemichannel; ER, endoplasmic reticulum; GJ, gap junction; G_qPCR, G-protein coupled receptor; IICR, inositol 1,4,5-trisphosphate-induced Ca^{2+} release; PIP2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate; IP_3R, inositol 1,4,5-trisphosphate receptor).

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).





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

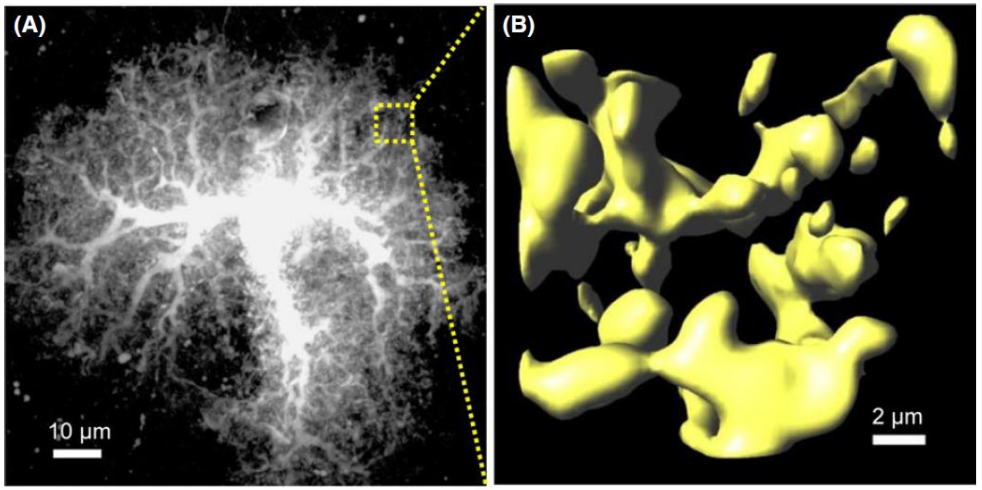
Emerging substances released by astrocytes

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

PAPs can invade the synaptic cleft



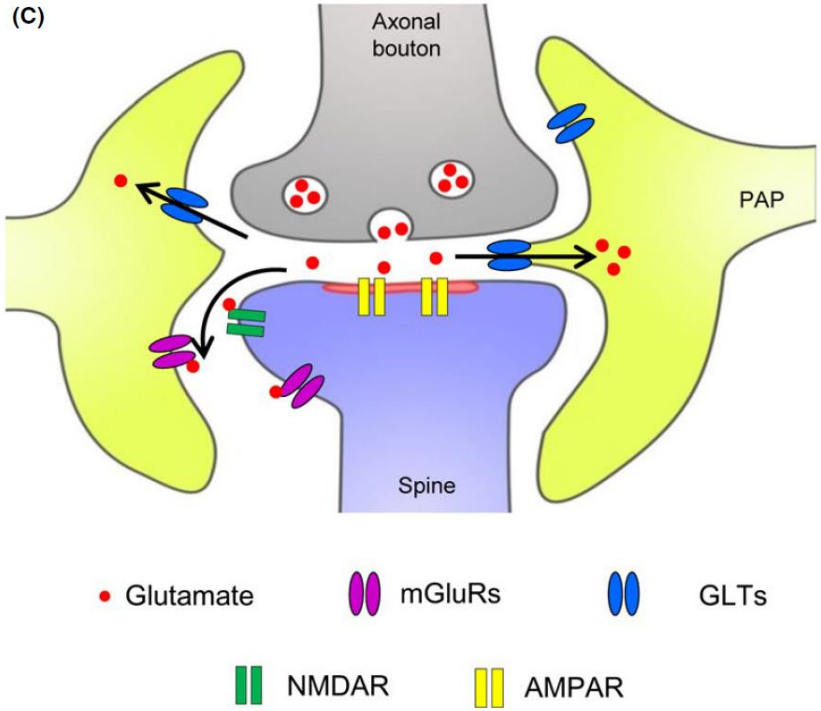


FIGURE 1 Protoplasmic astrocyte has very complex morphology with PAPs ensheathing the synapse. A, Representative confocal image of a protoplasmic astrocyte in the somatosensory cortex of adult mouse. B, Representative 3D reconstruction of astrocytic peripheral fine processes within a given ROI (5 μm × 5 μm × 5 μm). C, Cartoon of tripartite synapse, where PAPs approach or invade the synaptic cleft. The depth of astrocyte invasion controls the functional efficacy of GLTs which consequently affects the synaptic transmission. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GLTs, glial glutamate transporters, including GLT1 and GLAST; mGluRs, metabotropic glutamate receptors; NMDAR, N-Methyl-D-aspartic acid receptor; PSD, postsynaptic density. Images in A and B are provided by authors

Zhou et al, 2019
CNS Neuroscience & Therapeutics
DOI: 10.1111/cns.13123

PAPs plasticity = synaptic plasticity

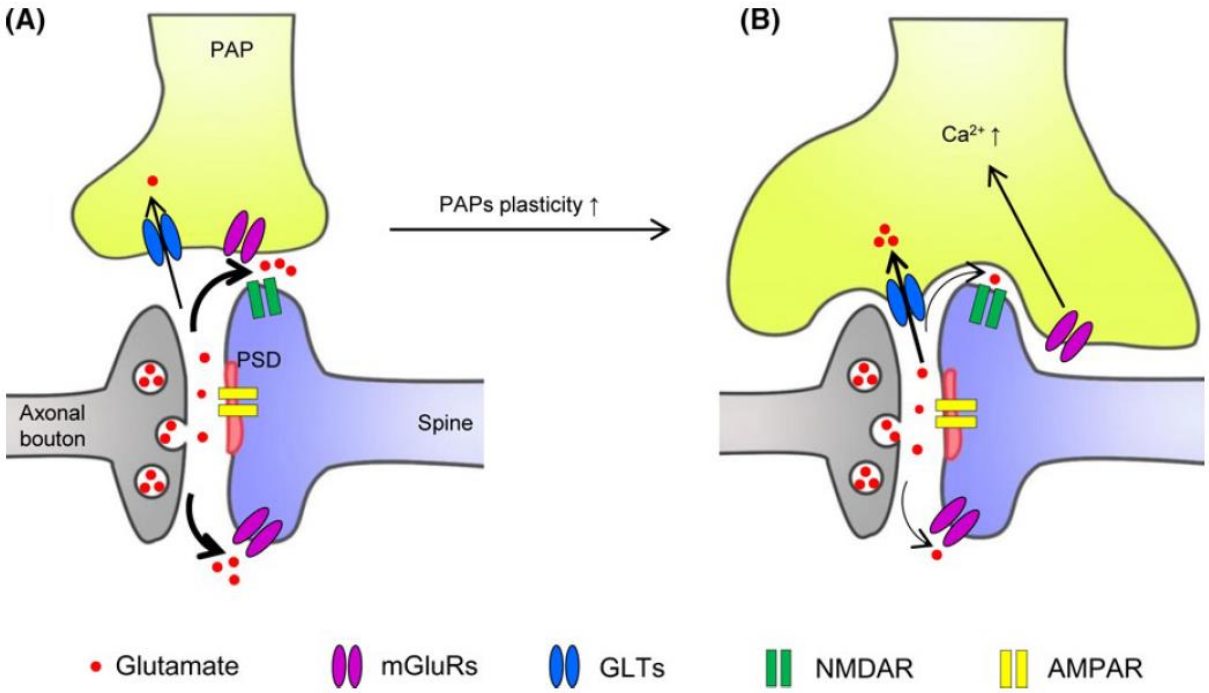
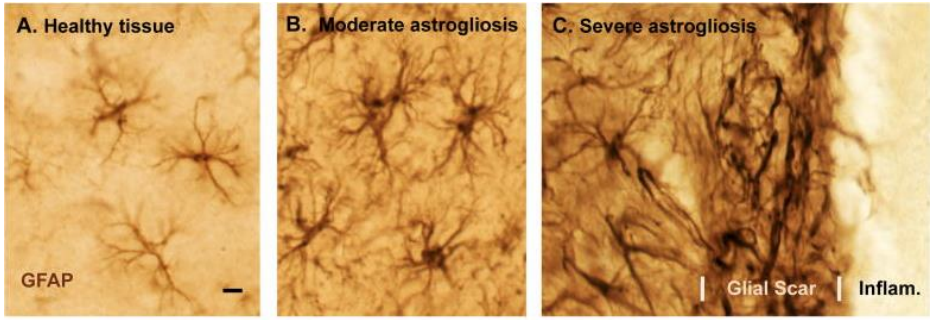


FIGURE 2 PAPs plasticity regulates the astrocytic coverage of synapse and synaptic transmission. A, A synapse with limited astrocytic coverage with low glutamate uptake by GLTs but high glutamate spillover which facilitates the activation of the extrasynaptic NMDAR and mGluRs. B, Increased PAPs plasticity, induced by mGluRs-mediated Ca^{2+} signals, enhances astrocytic coverage of synapse, with increased glutamate uptake by GLTs but decreased glutamate releasing into the extracellular space (ECS) and extrasynaptic activation. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GLTs, glial glutamate transporters, including GLT1 and GLAST; mGluRs, metabotropic glutamate receptors; NMDAR, N-Methyl-D-aspartic acid receptor; PSD, postsynaptic density

Zhou et al, 2019
CNS Neuroscience & Therapeutics
DOI: 10.1111/cns.13123

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



Acta Neuropathol (2010) 119:7–35

Different grades of reactive gliosis

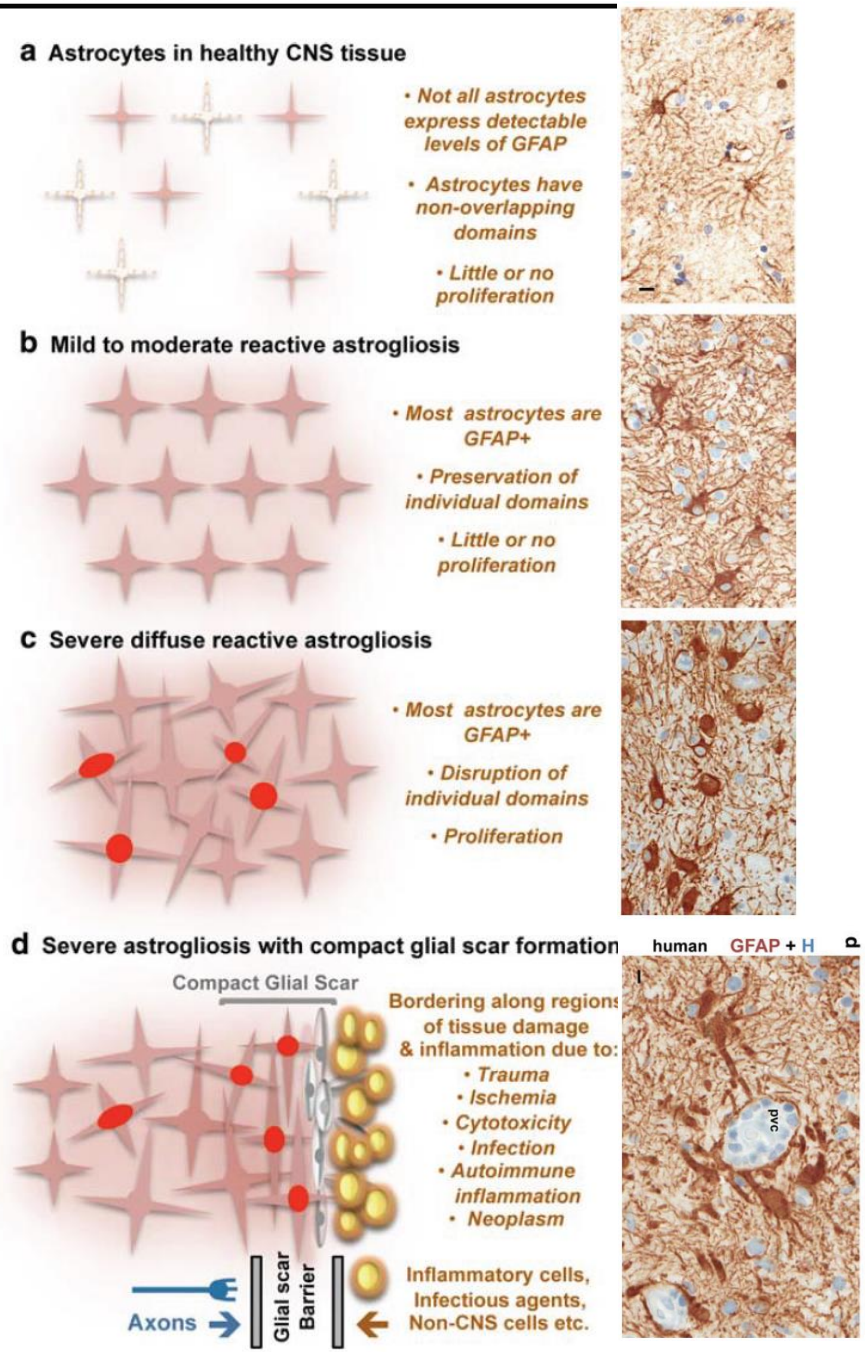


Fig. 4 Schematic representations that summarize different gradations of reactive astrogliosis. **a** Astrocytes in healthy CNS tissue. **b** Mild to moderate reactive astrogliosis comprises variable changes in molecular expression and functional activity together with variable degrees of cellular hypertrophy. 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

Morfological features of reactive astrocytes

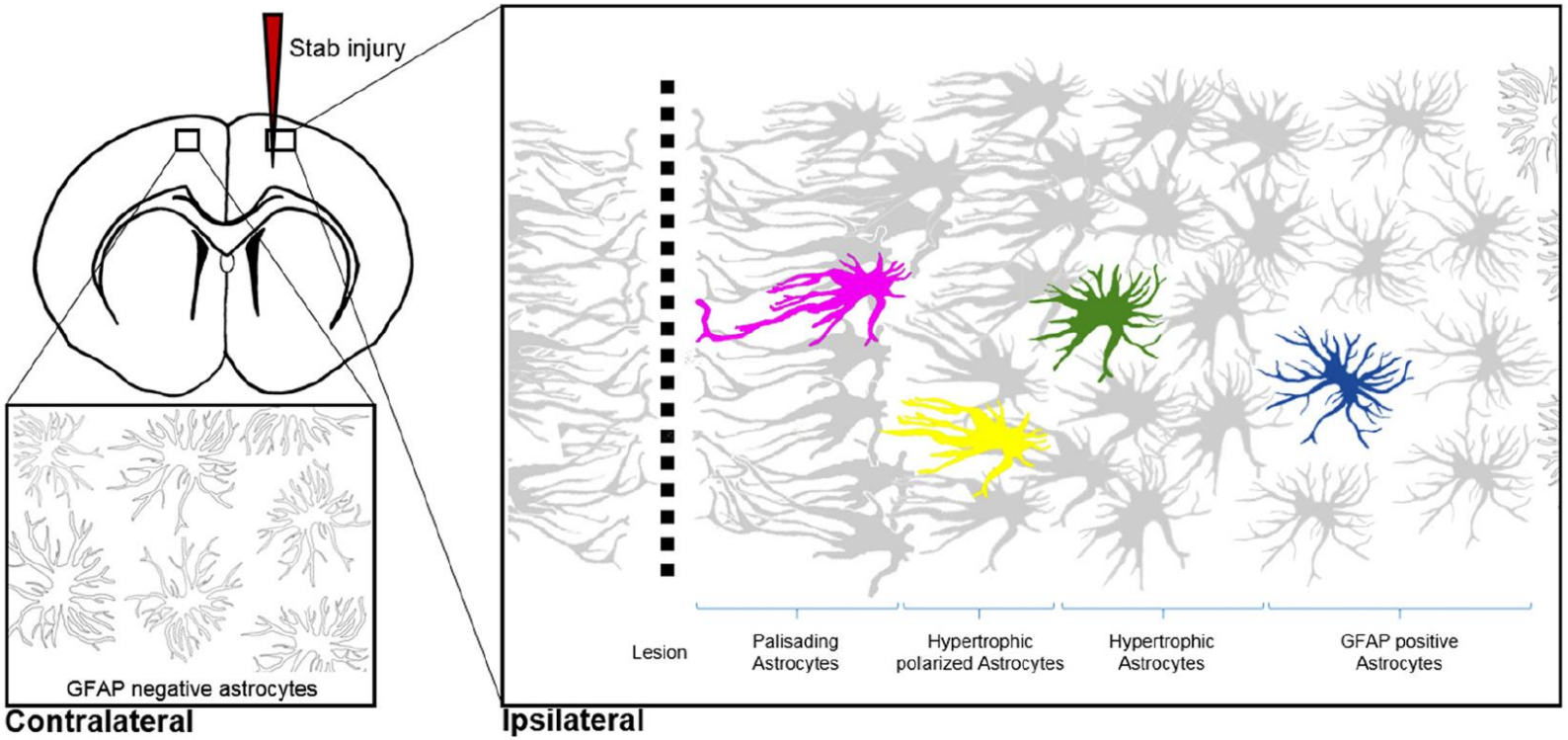
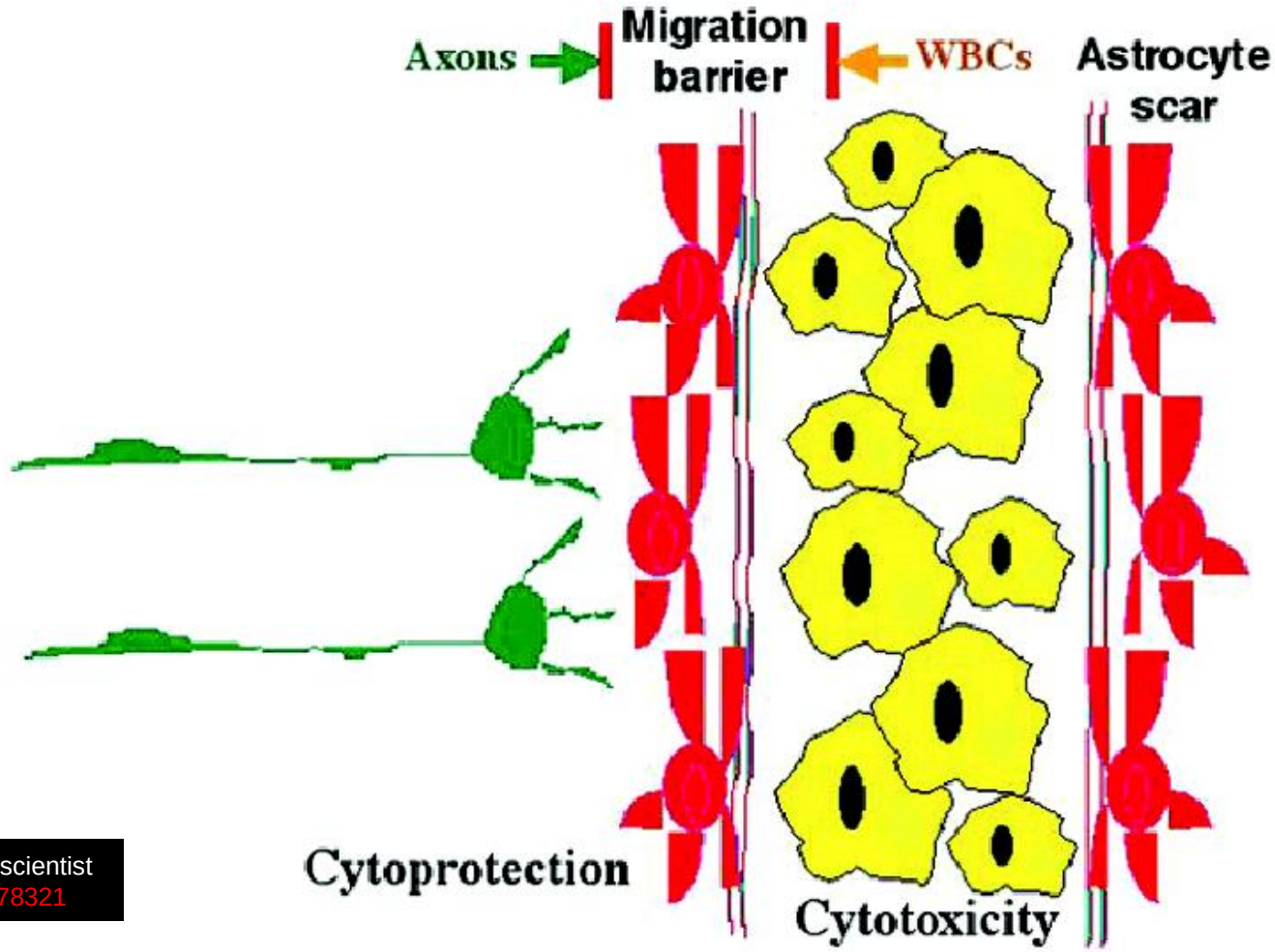


FIGURE 3 | Morphological hallmarks of reactive astrocytes in traumatic brain injury. Typical distribution of reactive astrocytes after a cortical stab wound in the mouse cortex 7 days after injury. Schematic drawings show the main processes of astrocytes, which contain high levels of GFAP (gray) upon injury. GFAP-positive astrocytes exhibit different morphologies depending on their distance from the lesion site: in the immediate vicinity (magenta), astrocytes polarize and extend long "palisading" processes. In the second row, multiple processes of the hypertrophic astrocytes (yellow) are orientated towards the injury site; with increased distance, astrocytes possess hypertrophic cell bodies and primarily main processes (green). Finally, reactive astrocytes at the border of the unaffected parenchyma upregulate GFAP but show no signs of hypertrophy (blue). Note that cortical astrocytes in the uninjured contralateral hemisphere sparsely express GFAP, as indicated with silhouettes (bottom left image).

Schiweck et al., 2018
Frontiers in Cellular Neuroscience
doi: 10.3389/fncel.2018.00261

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.

Astrocytes: Emerging Therapeutic Targets in Neurological Disorders

Chiara F. Valori,[1] Giulia Guidotti,[2] Liliana Brambilla,[2] and Daniela Rossi[2,*]

Trends in Molecular Medicine, September 2019, Vol. 25, No. 9 https://doi.org/10.1016/j.molmed.2019.04.010

Key Figure

Charting the Path to Astrocytes

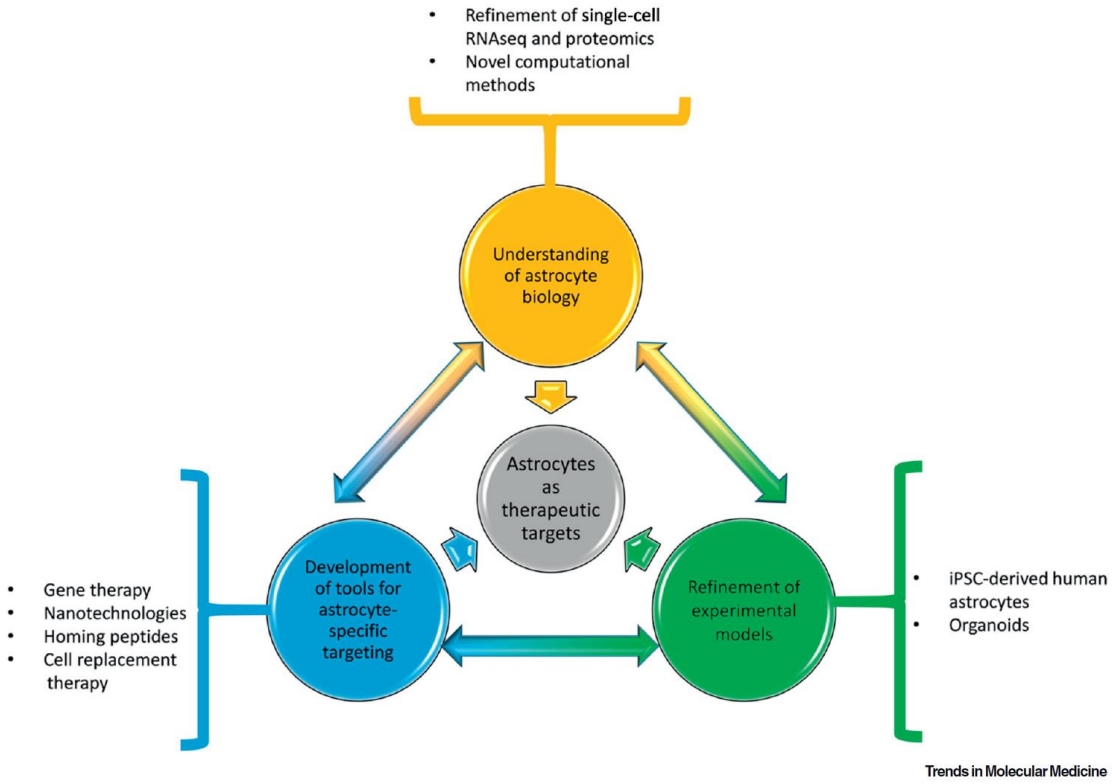


Figure 1. Many proof-of-principle studies indicate that targeting astrocytes might be crucial to successfully tackling diverse neurological conditions. To reach this objective, we identify multiple lines of investigation, falling into three major interconnected research themes: gaining a thorough understanding of astrocyte biology in humans and rodents (top, yellow circle); developing faithful experimental models to be used for mechanistic investigations and drug screening (right, green circle); and refining tools for selective and specific delivery of therapeutics to astrocytes (left, blue circle). Abbreviation: iPSC, induced pluripotent stem cell.

Highlights

Single-cell RNA sequencing is redefining the concept of astrocyte diversity at the molecular level. The clarification of astrocyte heterogeneity has important implications for the comprehension of central nervous system (CNS) physiopathology.

Astrocytes have emerged as primary players in the mechanisms of neurodegeneration and the progression of several neurological disorders.

Viral vectors, nanoformulations, and peptide-based treatments for the selective targeting of harmful pathways in astrocytes are being developed and hold potential for the treatment of neurological disorders in preclinical models

Engrafted healthy astrocytes can survive in a pathological milieu and improve the phenotype of animal models of CNS disorders. This raises the expectation of astroglial cell transplantation as a valuable therapeutic tool.

Outstanding questions on Astrocytes

What are the scientific questions we need to answer before we can use astrocytes as therapeutic targets?

- How functionally similar are human and rodent astrocytes?
- Does each subpopulation of astrocytes react differently to specific insults (e.g., chronic neurodegenerative diseases, viral infections, traumatic injury, stroke, or demyelinating disorders) or is there a specific type of stimulus that drives the switch to a specific phenotype?
- Which astrocyte signaling pathways are affected in different CNS disorders?
- Is the transplant of healthy astrocytes an effective strategy to counteract disease progression in different neurodegenerative conditions?
- Can astrocytes transdifferentiate into functional neurons in vivo and effectively integrate into the correct brain circuitry?