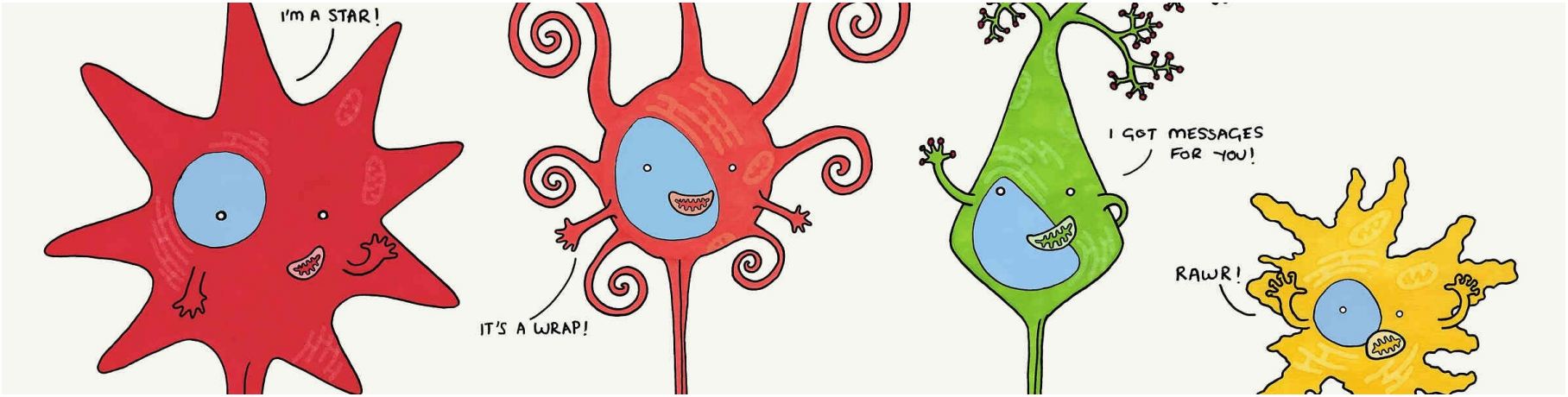
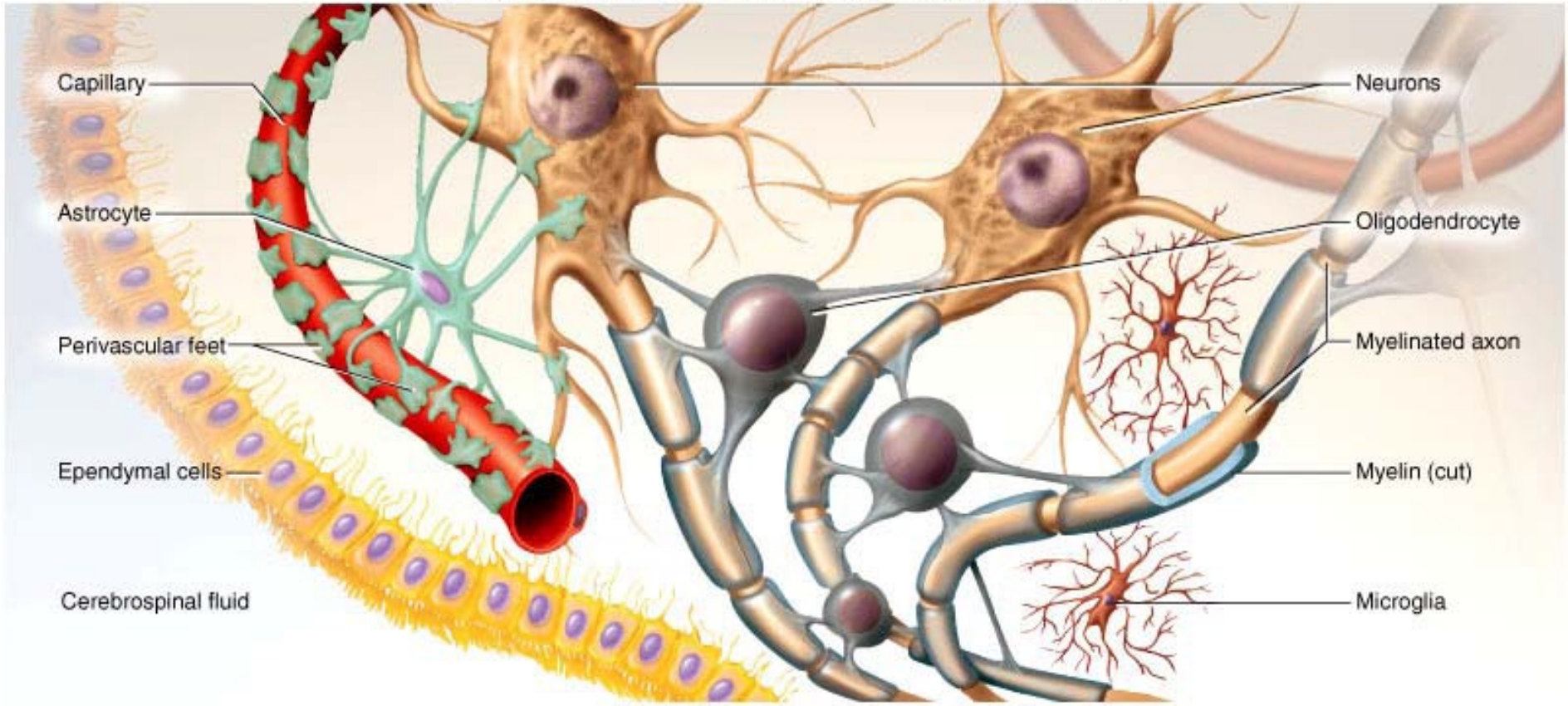
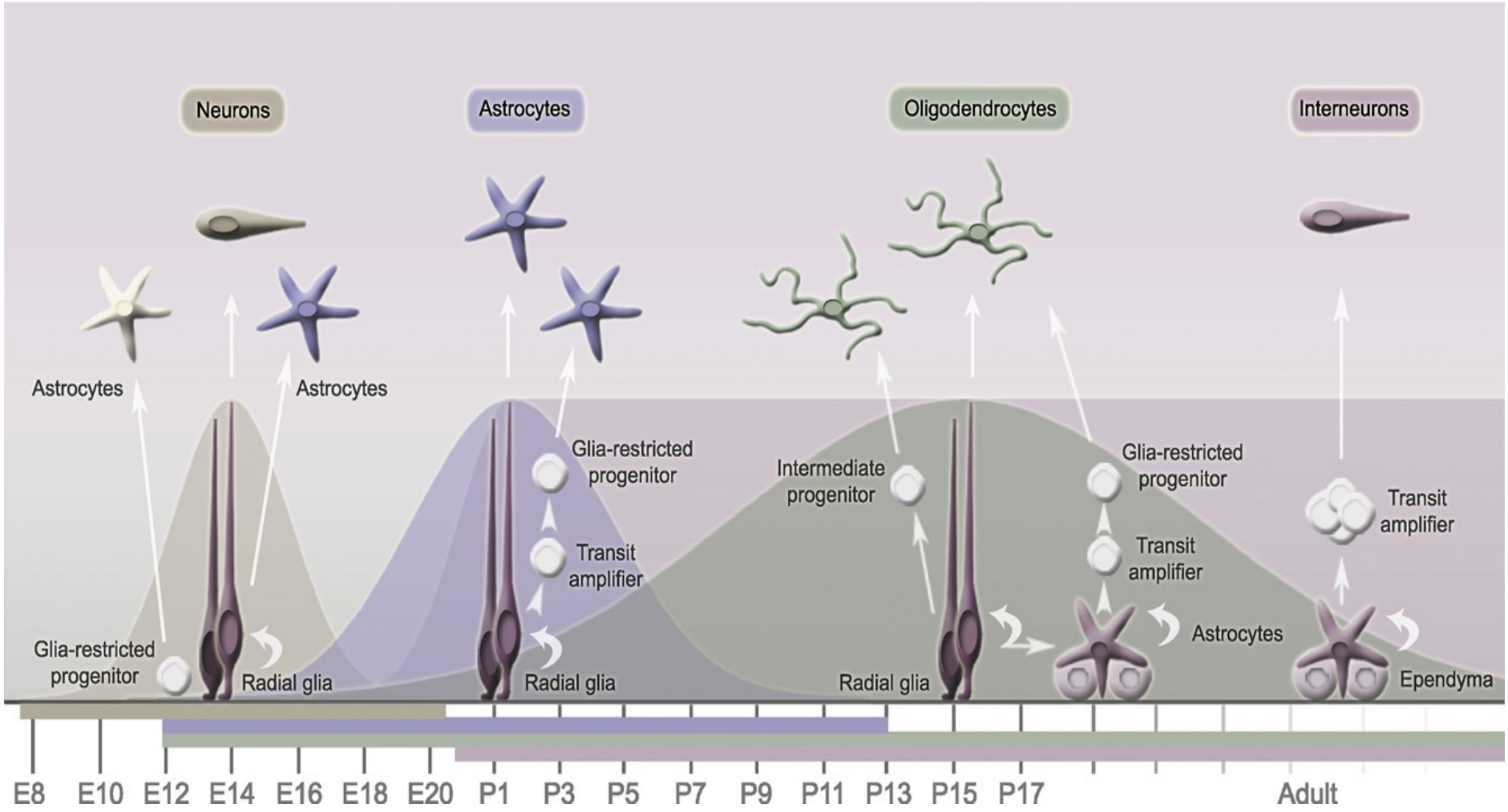


GLIA

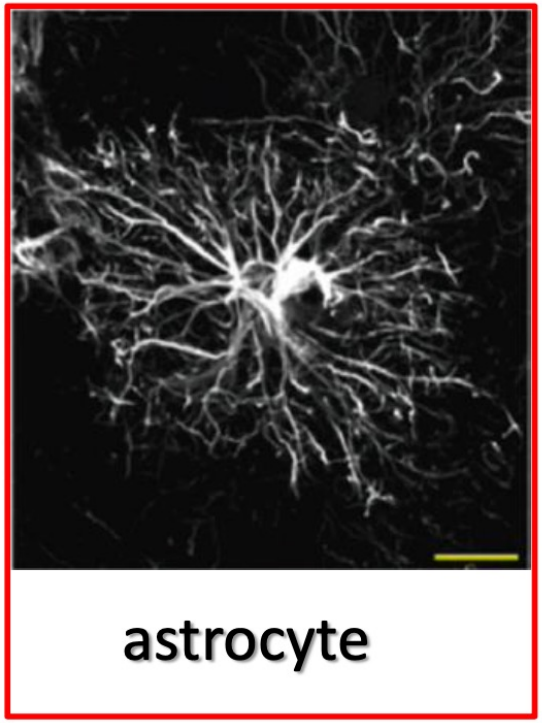
Neurocentric vision…..



Developmental origin

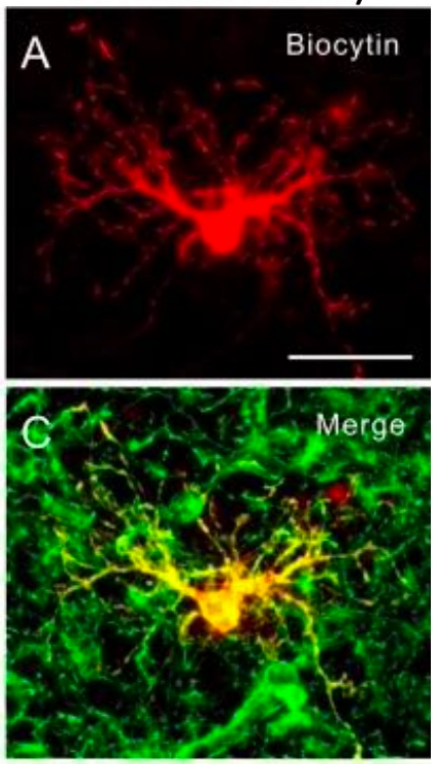


Non-myelinating glia in the CNS: ASTROCYTES, MICROGLIA, OPCs

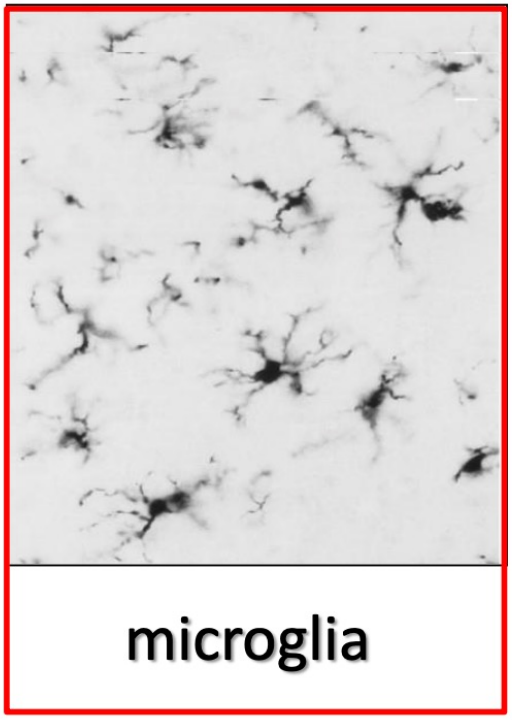


astrocyte

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)

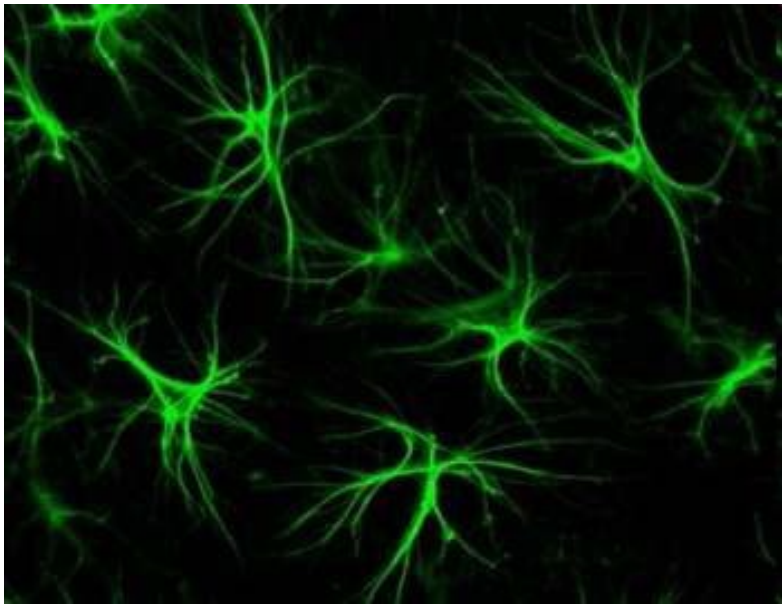
Types of glial cells: astrocytes

- housekeeping functions necessary to maintain neuronal function,
- actively shape synaptic function
- neural precursors in adult neurogenic regions

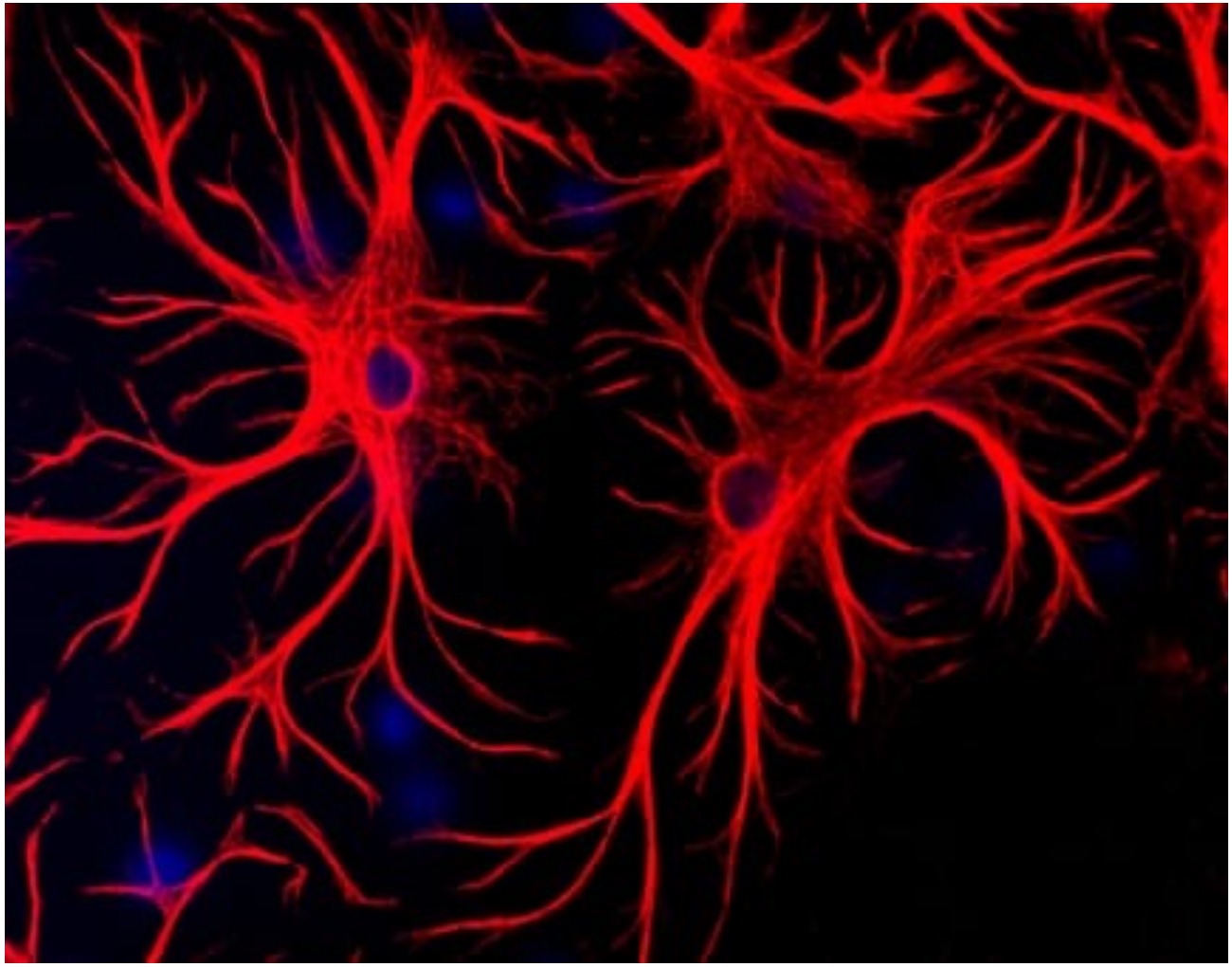
Astrocytes regulate extracellular environment

- Produce and release neurotrophic factors (NGF, CNTF, IGF, TGFβ, βFGF)
- Store glycogen as an energy source
- Regulation of composition of the extracellular space(K^+, ATP, H^+)
- Uptake and degradation of glutamate
- Active role in communication
- spontaneous Ca^{2+} waves
- glutamate release → neuronal activation
- Promote synapse formation and function

Glial fibrillary acidic protein (GFAP)



Glial Fibrillary Acidic Protein (GFAP), The Most Popular Astrocyte Marker



GFAP, a class-III intermediate filament, is a 50kDa protein which is found in the mature and developing astrocytes

Astrocytic Morphology

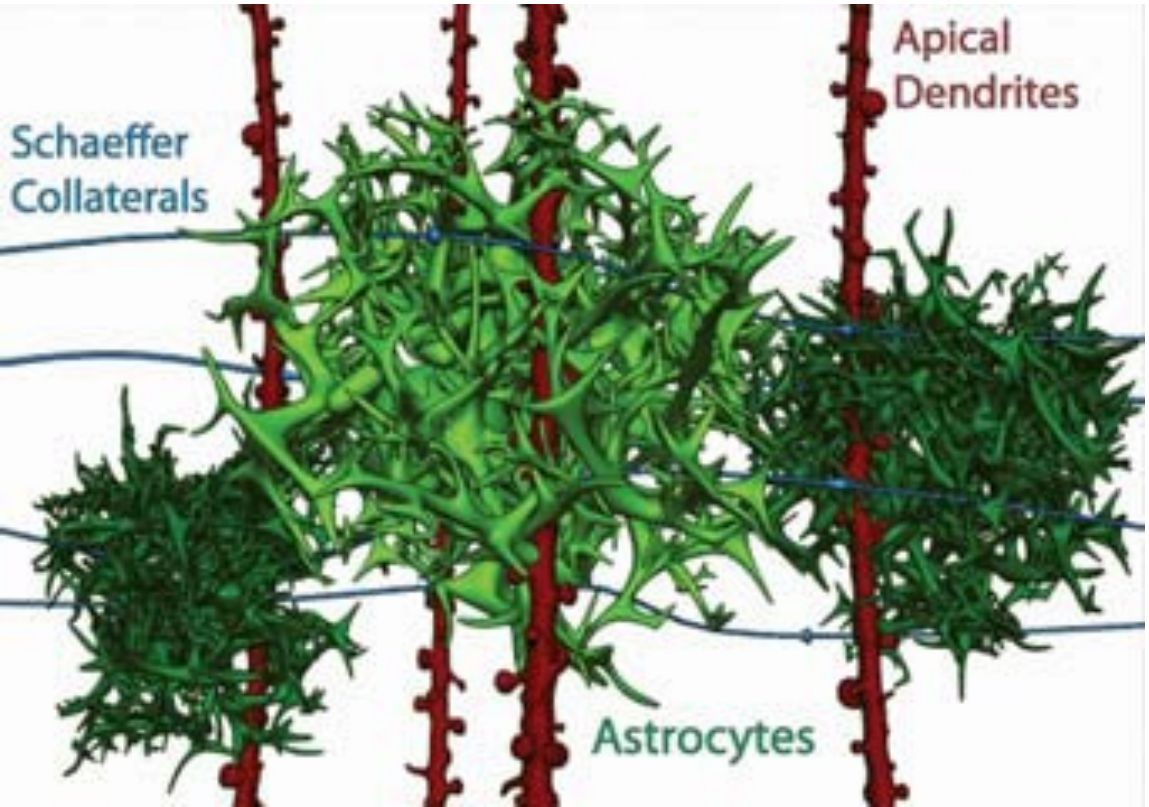
Largest cell bodies among glia cells

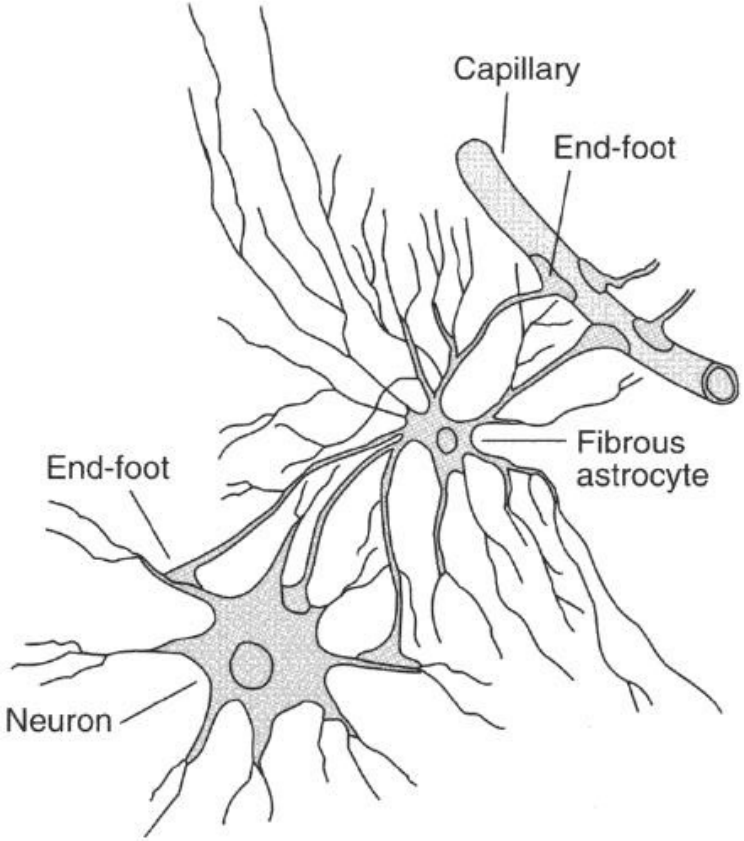
Many processes “star-like”

Protoplasmic astrocytes occupy restricted and independent territories

‘End feet' contact capillary endothelium

Line the exterior surface of the CNS, (glial limitans)





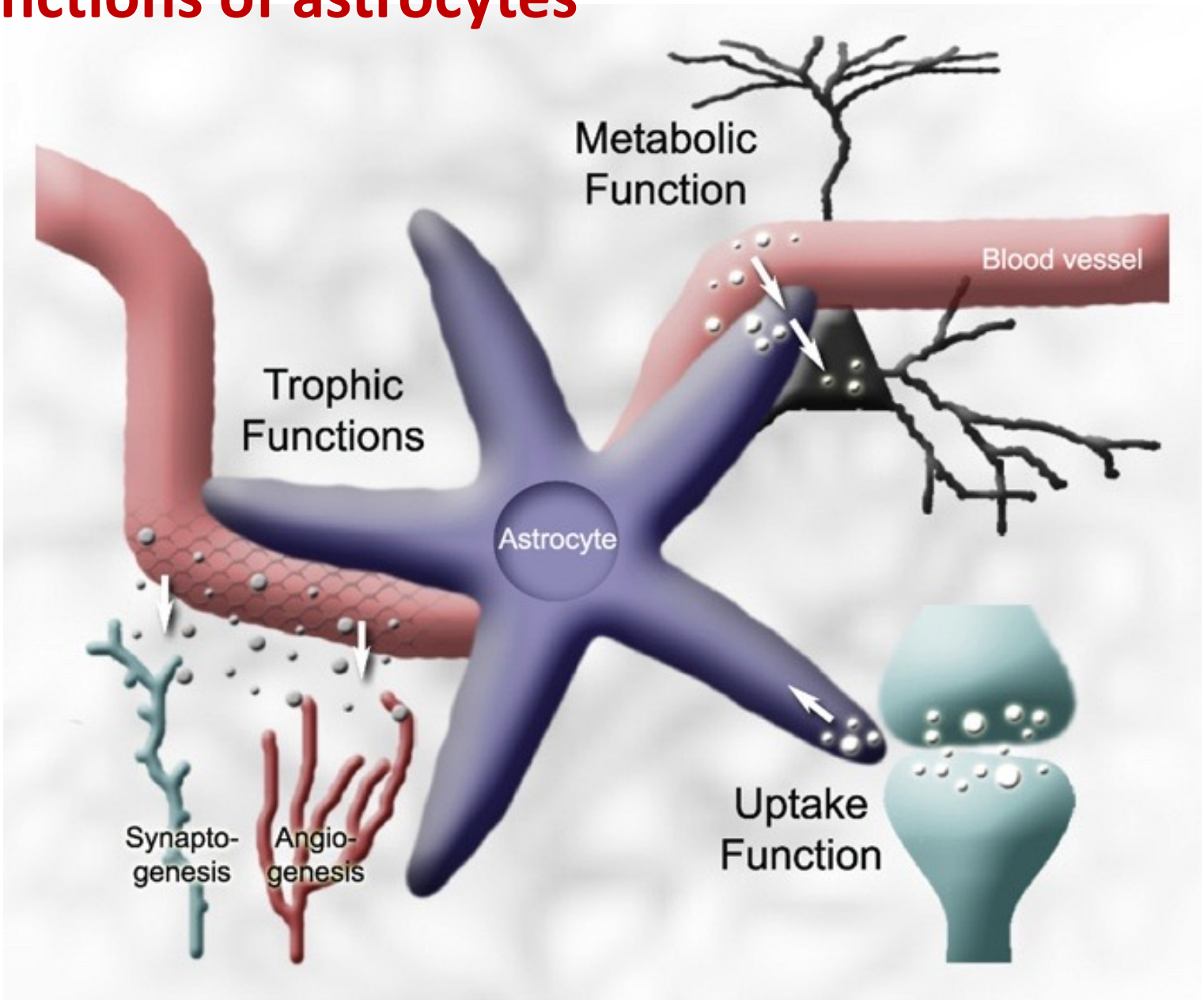
Functions of astrocytes

- Synthesis of extracellular matrix proteins, adhesion molecules and trophic factors
- Angiogenesis, BBB
- Extracellular ion buffering
- Glutamate and GABA uptake
- Metabolic support
- Detoxification and immunity

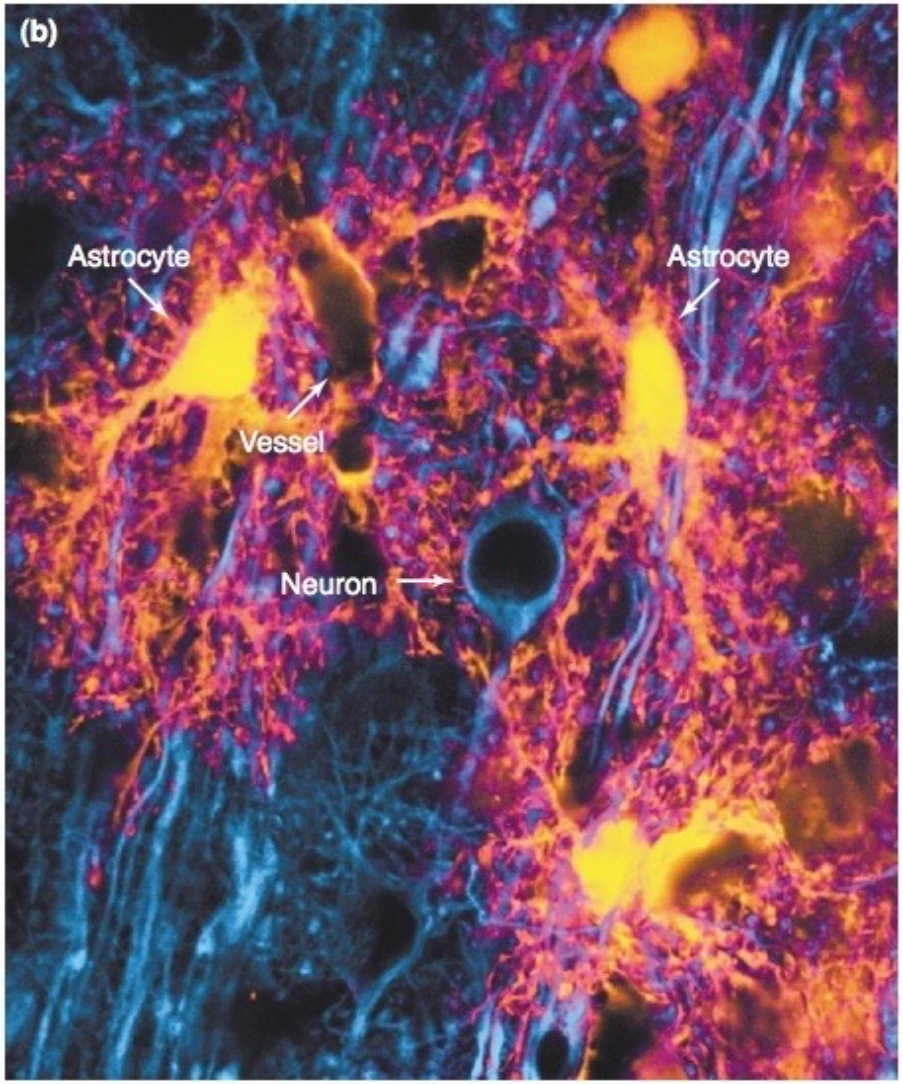
Functions of astrocytes

- Sensing neuronal activity
- Calcium dynamics
- Neurovascular unit
- Control of vascular tone
- Dynamic control of synaptic structure and function

Functions of astrocytes



Organization of Neuroglia tissue



Nedergaard et al., TINS 26, 523 (2003)

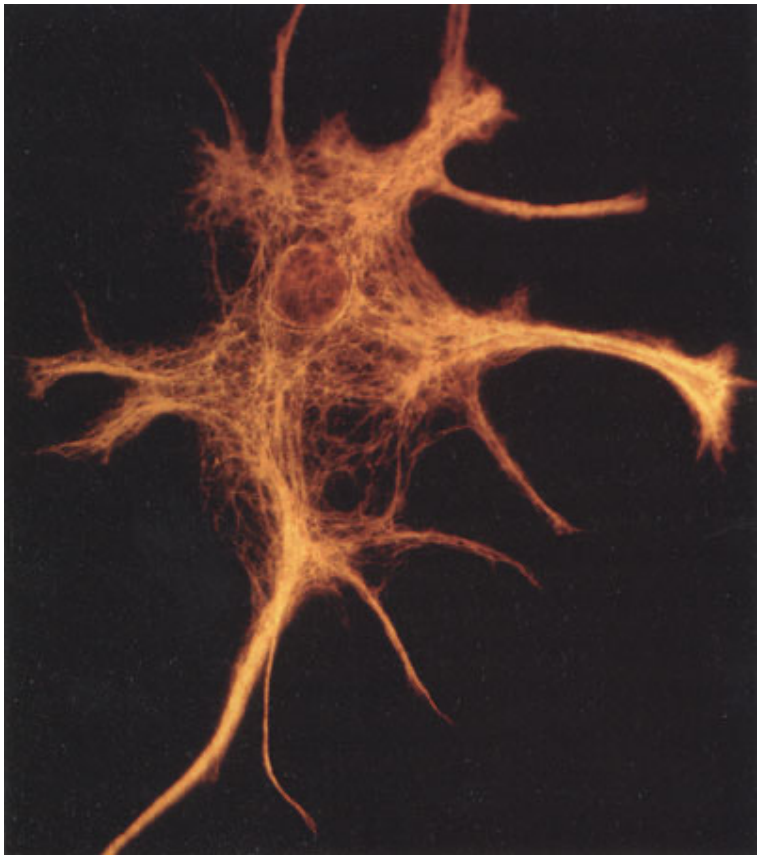
Astrocytes

– are more numerous than neurons

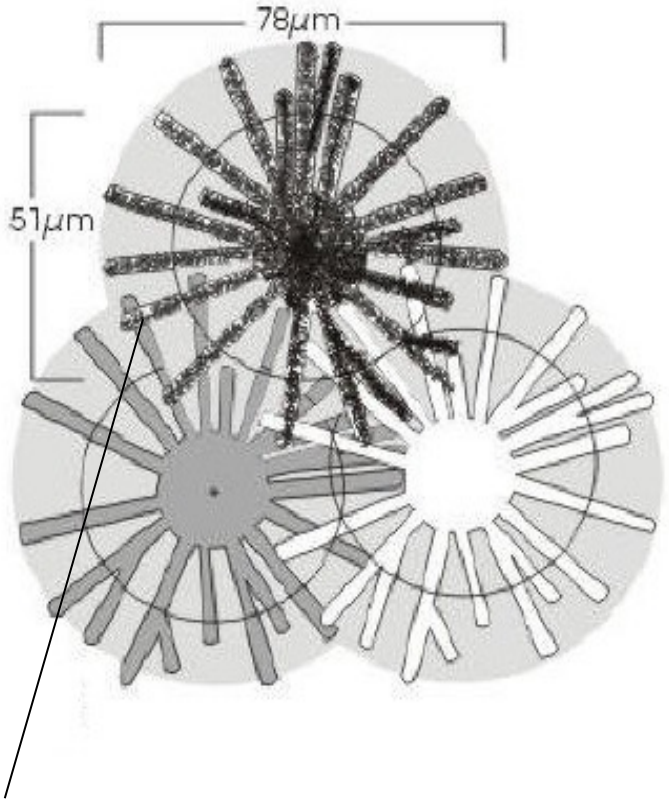
– have processes which associate with synapses

– seem to be required for various aspects of neural development, maintenance and neural dynamics

Astrocytes



V. Parpura, UC-Riverside
glial fibrillary acidic protein (GFAP) tagged with antibody.



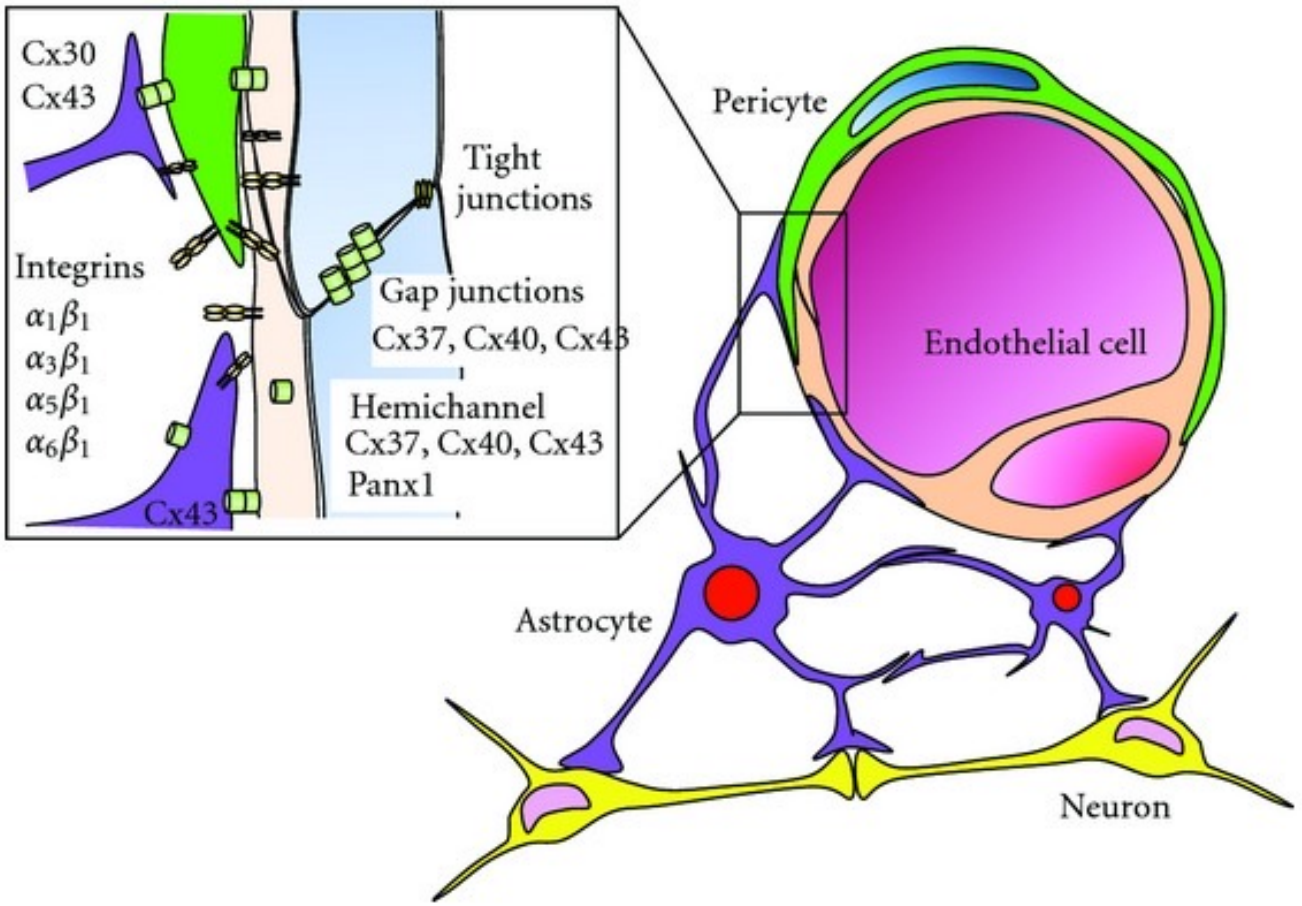
Astrocytes are connected by gap junctions thereby forming a syncytium that is able to propagate signals for large distances

Gap junctions

- Homocellular
- Heterocellular

Astrocytes are high

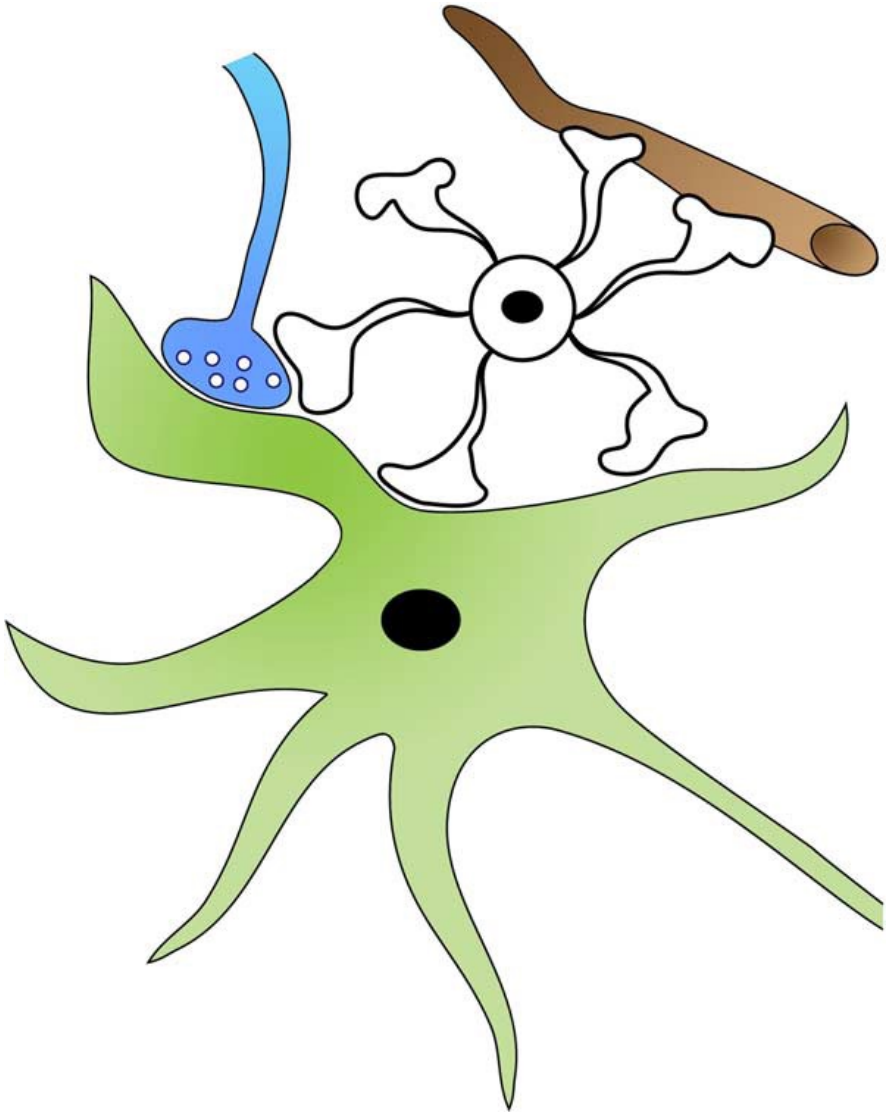
Panglial syncytium



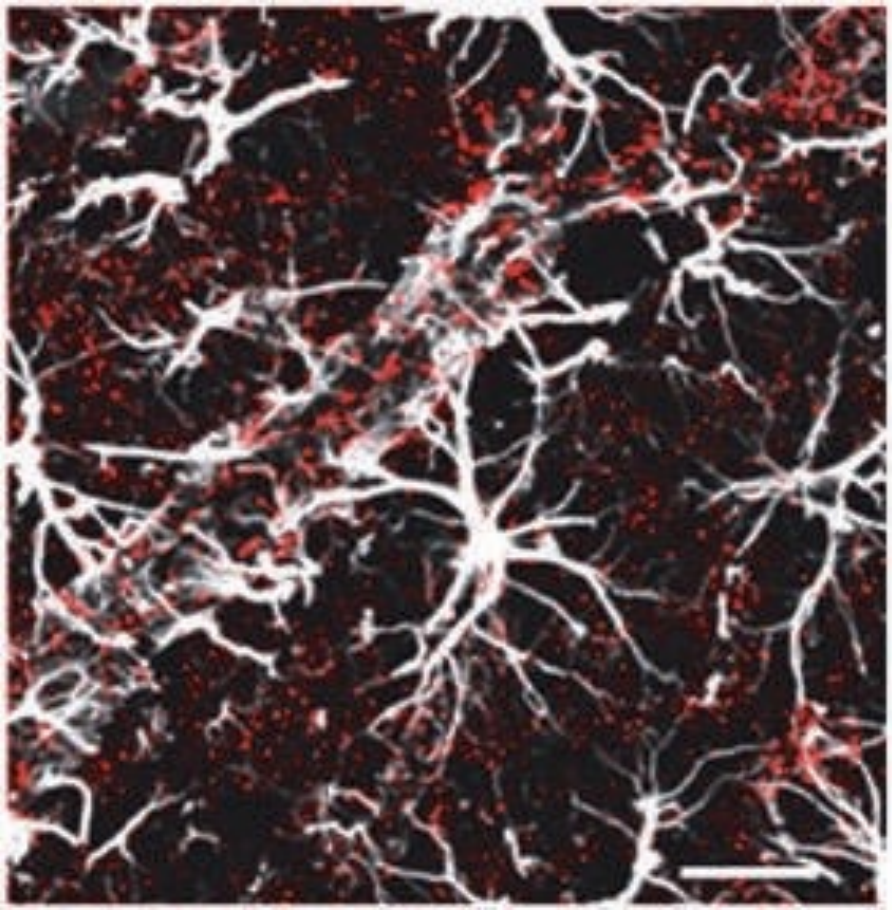
Gap junctions

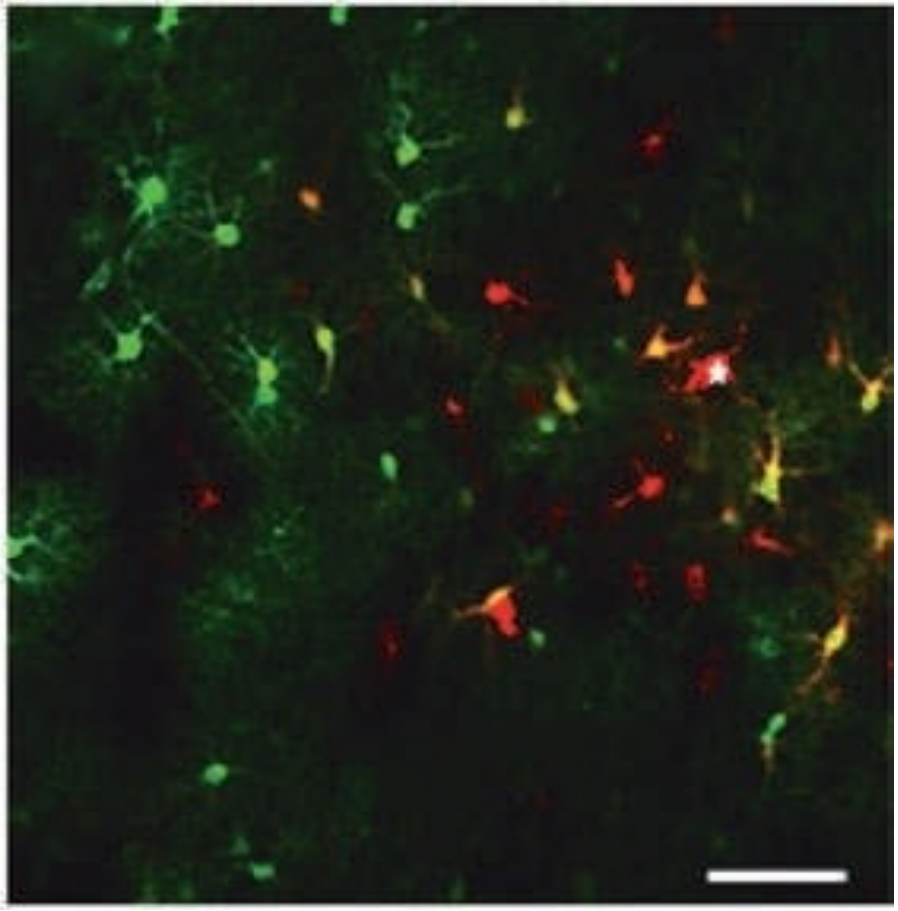
Astrocytes contact virtually every cell component in the brain

- Other astrocytes (gap junctions)
- Ependymal cells
- Neurons (somas, processes, synapses
- Oligodendroglia
- Capillary endothelial cells

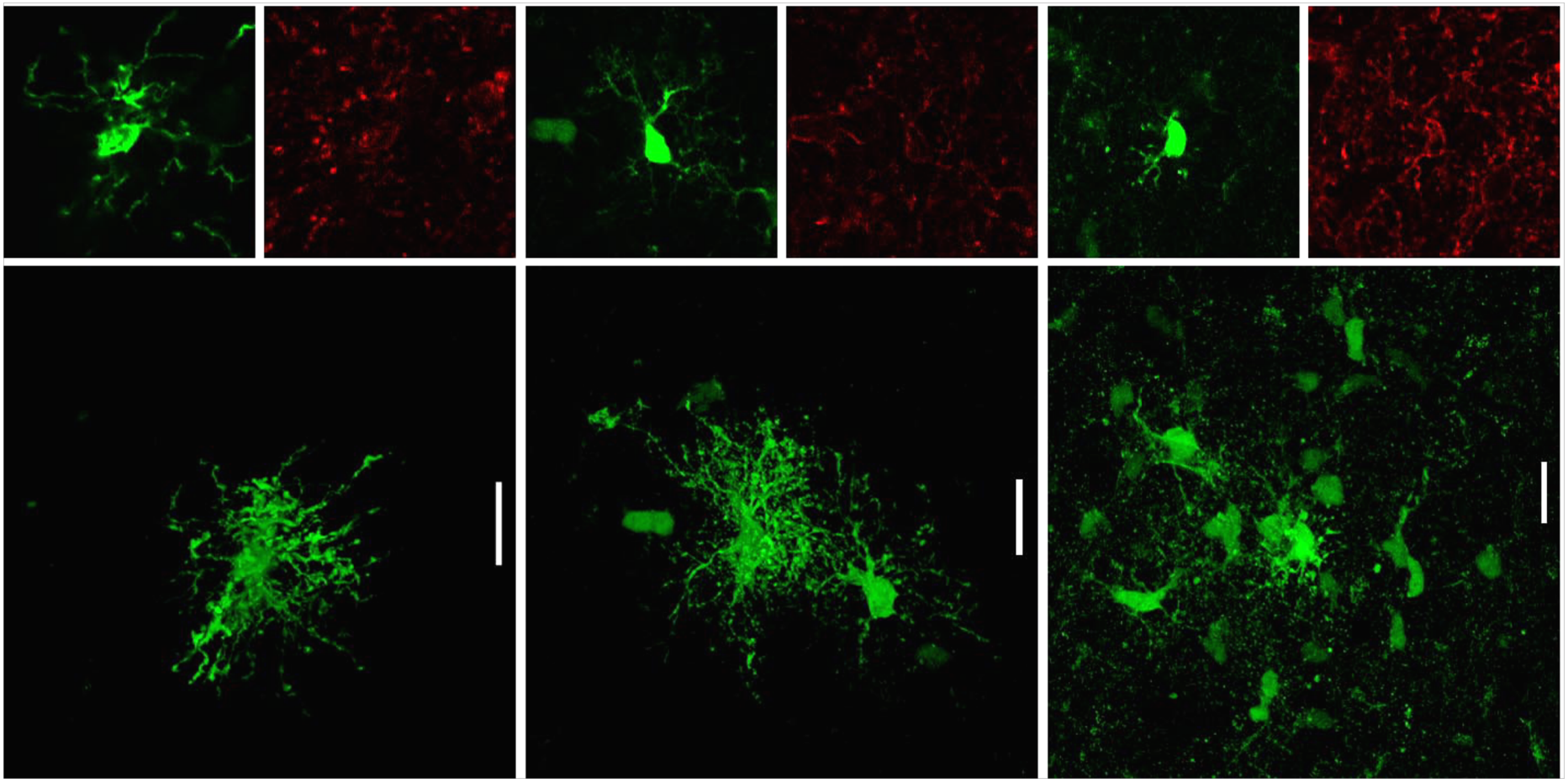


Gap junctions

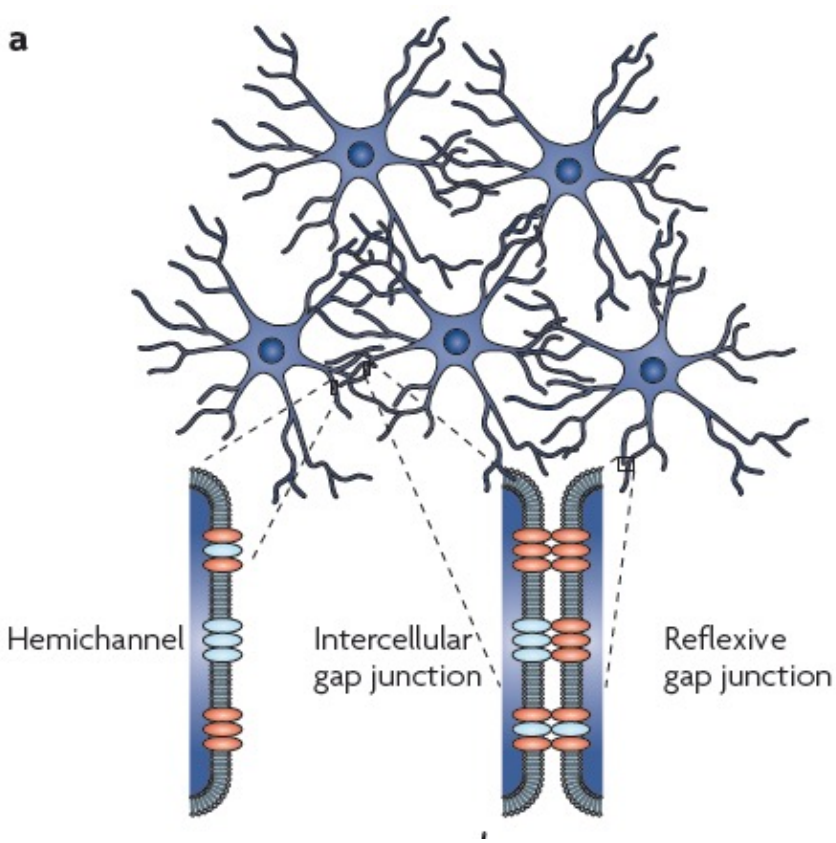


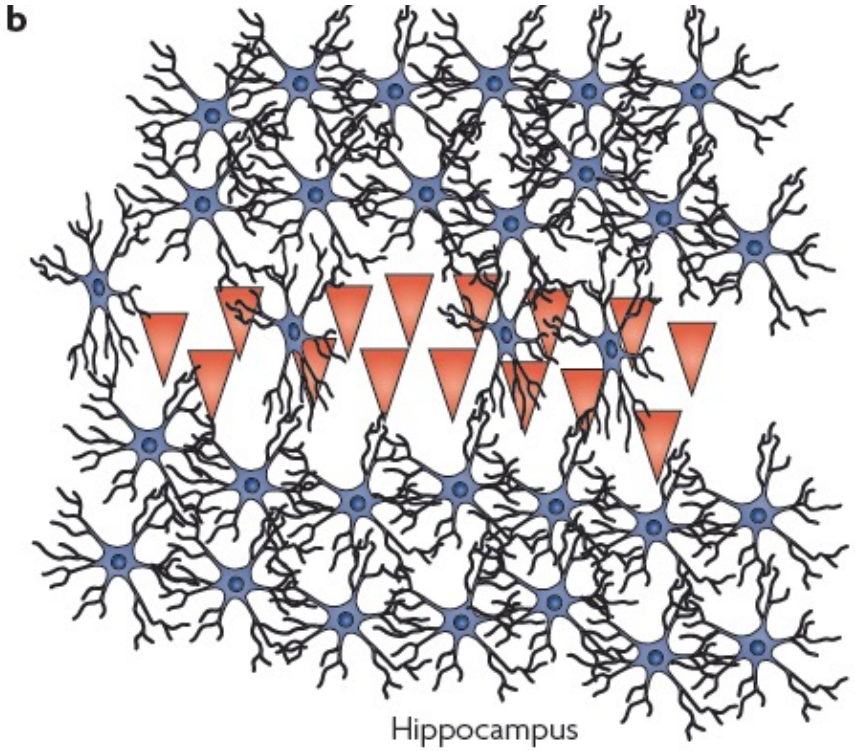


Gap junctions



Astrocyte network





protoplasmic astrocytes of the grey matter occupy very restricted and independent spatial territories.

Astrocytes as Modulators of Synaptic Information Transfer

Extracellular ion buffering

Extracellular ion buffering

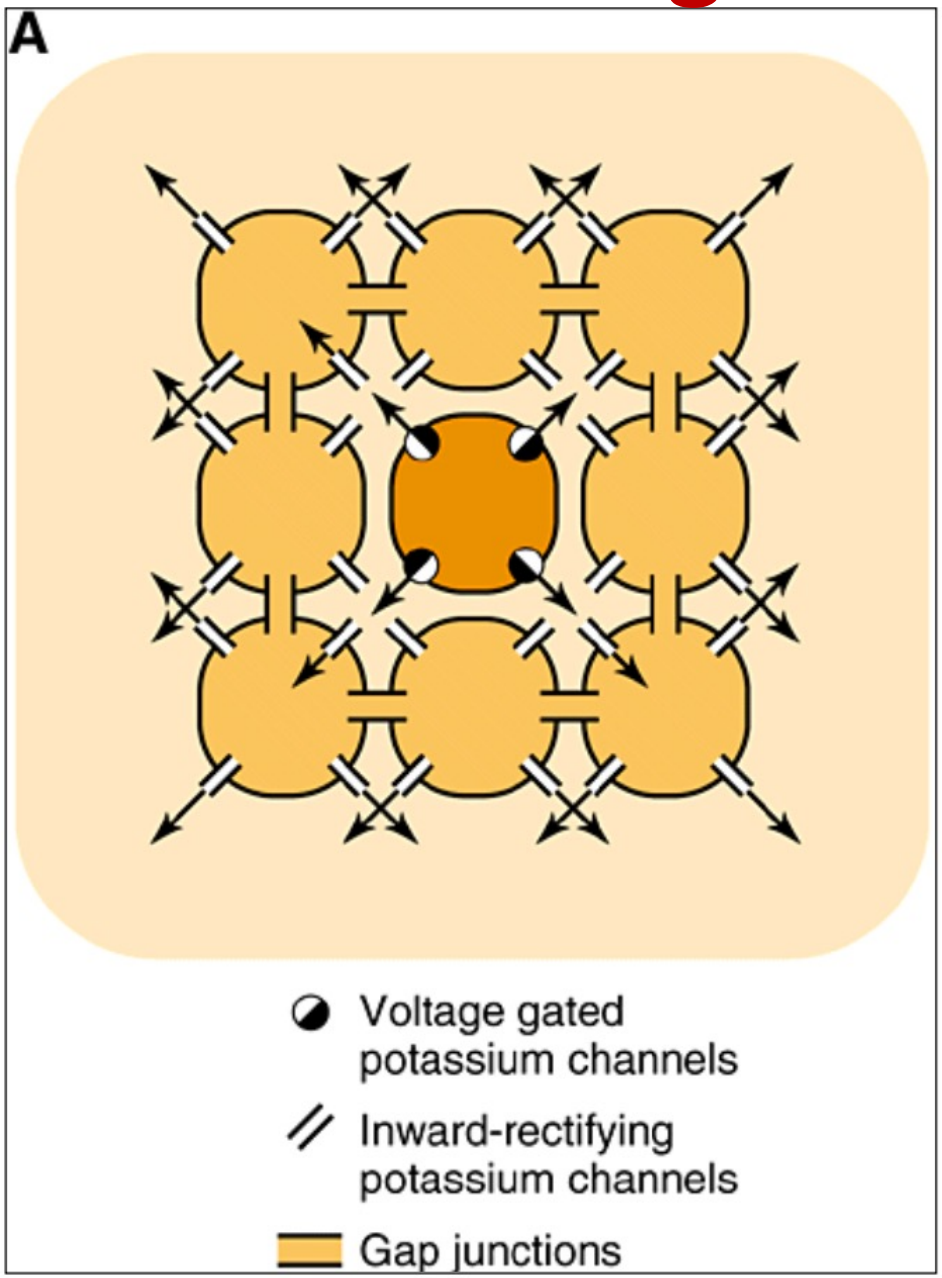
- Astrocytes buffer excess K^+ ions (intense neuronal activity)
- Distribute ions in the syncytium (spatial buffering)
- Extrude them into the interstitium or perivascular space (then to blood flow)

- Passive transport (K_{IR}, transporters)
- Active transport (Na/K ATPase)

Extracellular ion buffering

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.

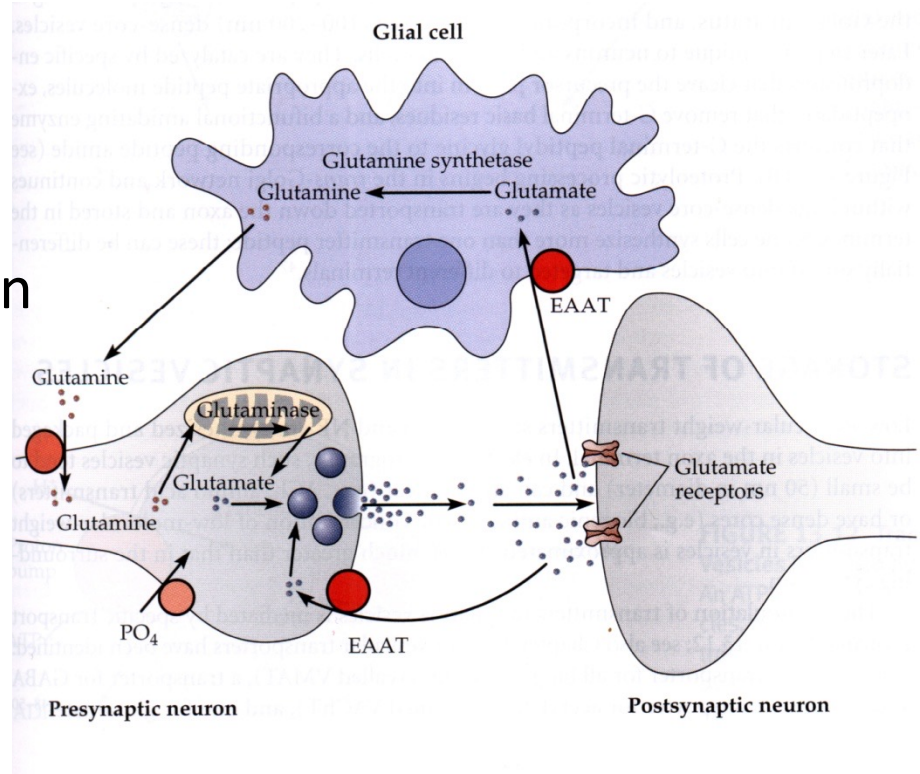


Regulation of extracellular glutamate concentration

Regulation of extracellular glutamate concentration

- Prevent contamination by non synaptic glutamate
- Remove glutamate form synaptic cleft
- Provide rapid replenishment

- Activated by synaptic glutamate
- Transmitter time course
- Reliability of synaptic transmission



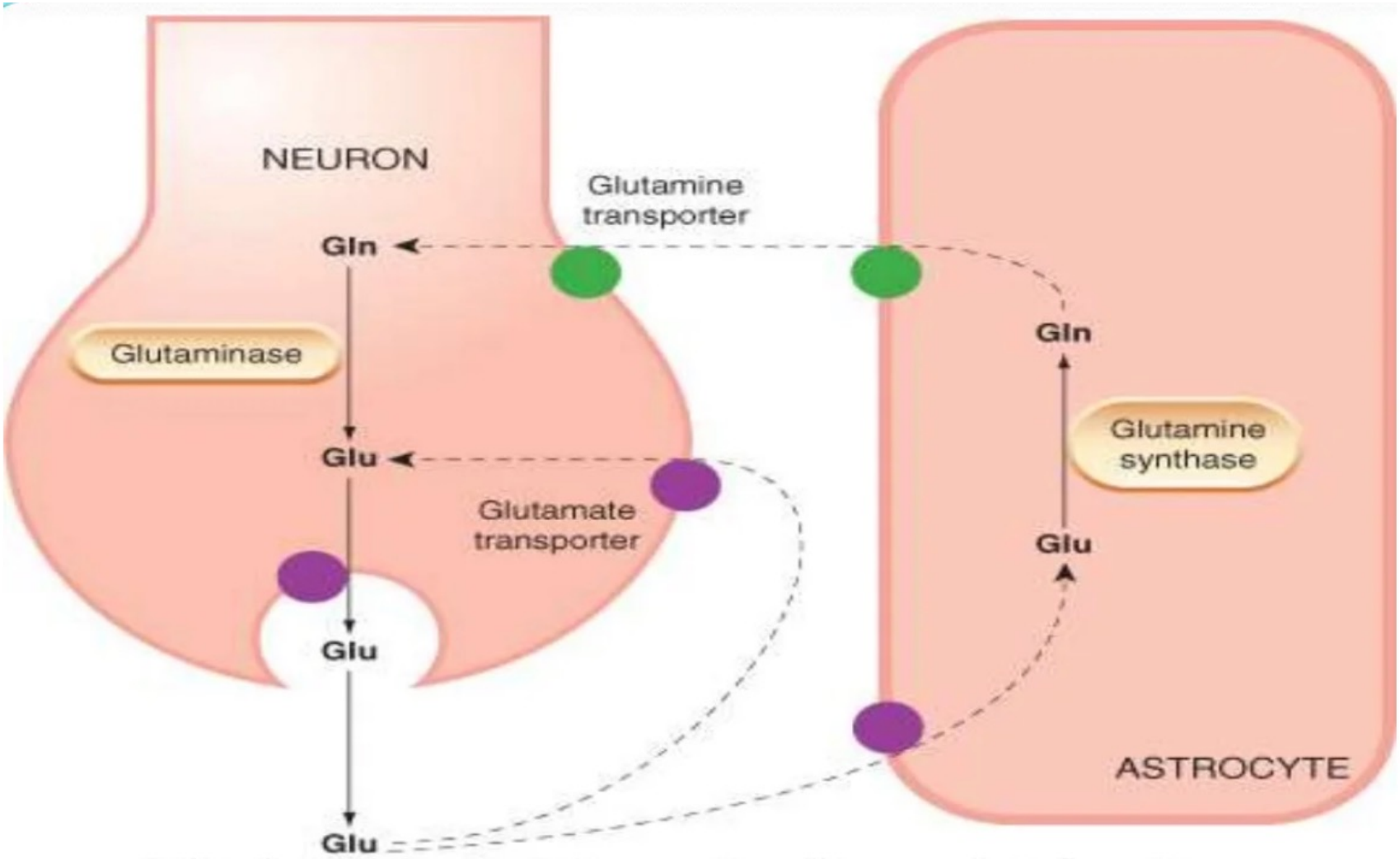
Syntesis of glutamate at synapses

- Given the excitatory effects of glutamate, it is excluded from the brain by BBB i.e, Blood Brain Barrier is impermeable to Glutamate.
- Thus, glutamate in the brain must be synthesised de novo from Glucose,

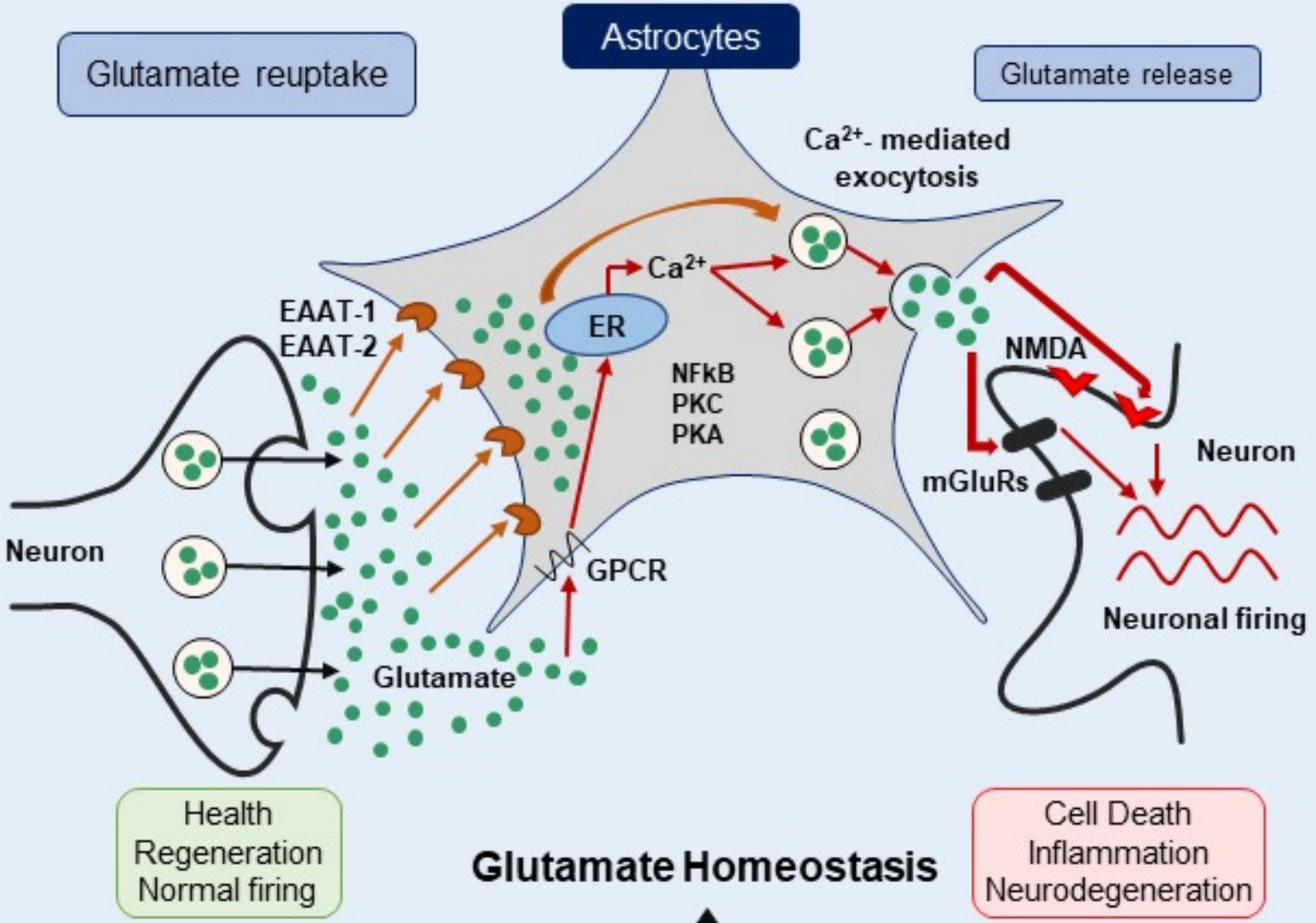
  TCA → Alpha Ketoglutarate → Glutamic acid (via transamination)
- Reuptake to storage vessels,

  20% of glutamate turnover through 'glutamate transporter' & 40% through 'glutamine cycle'.

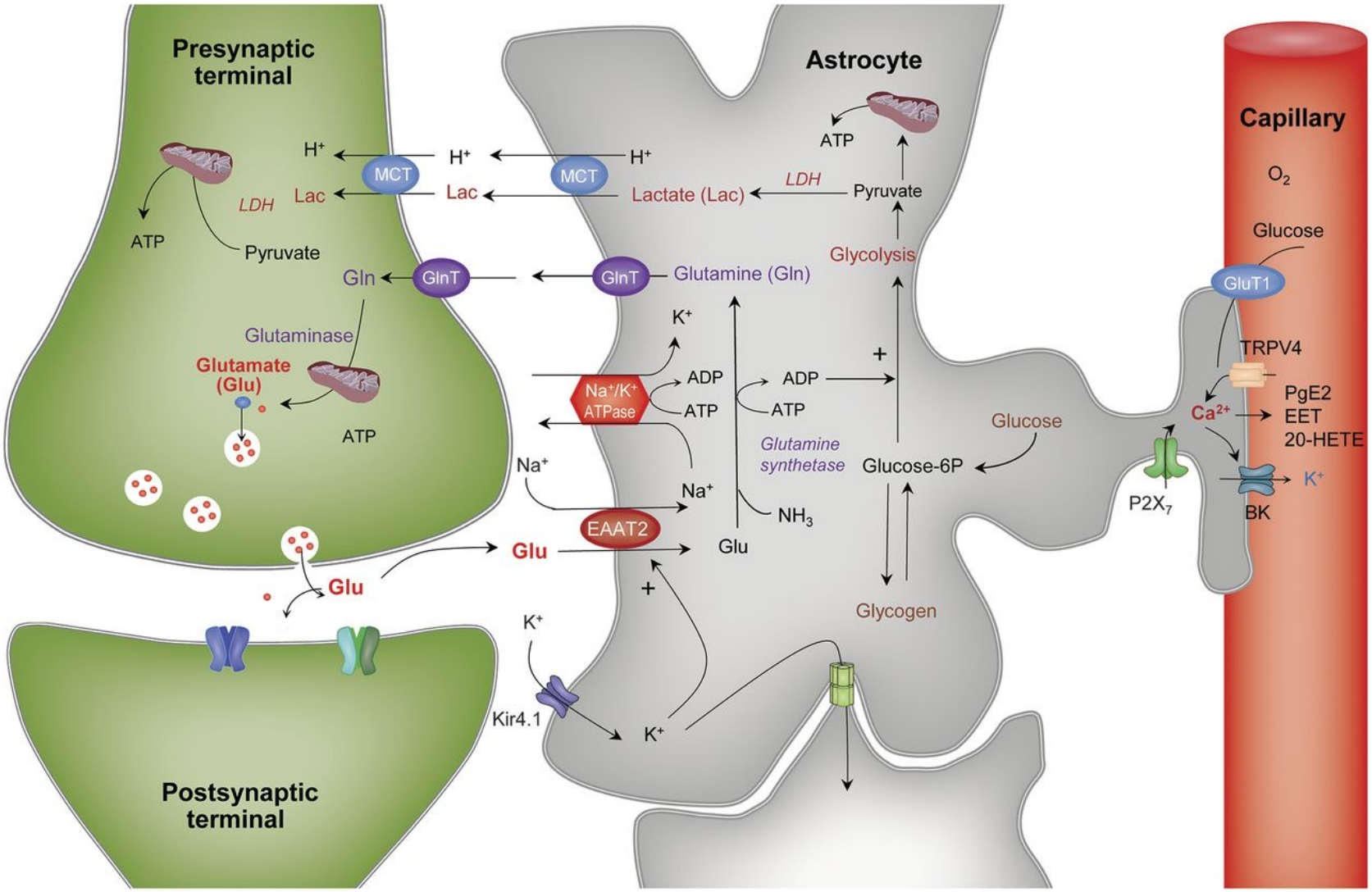
Regulation of extracellular glutamate concentration



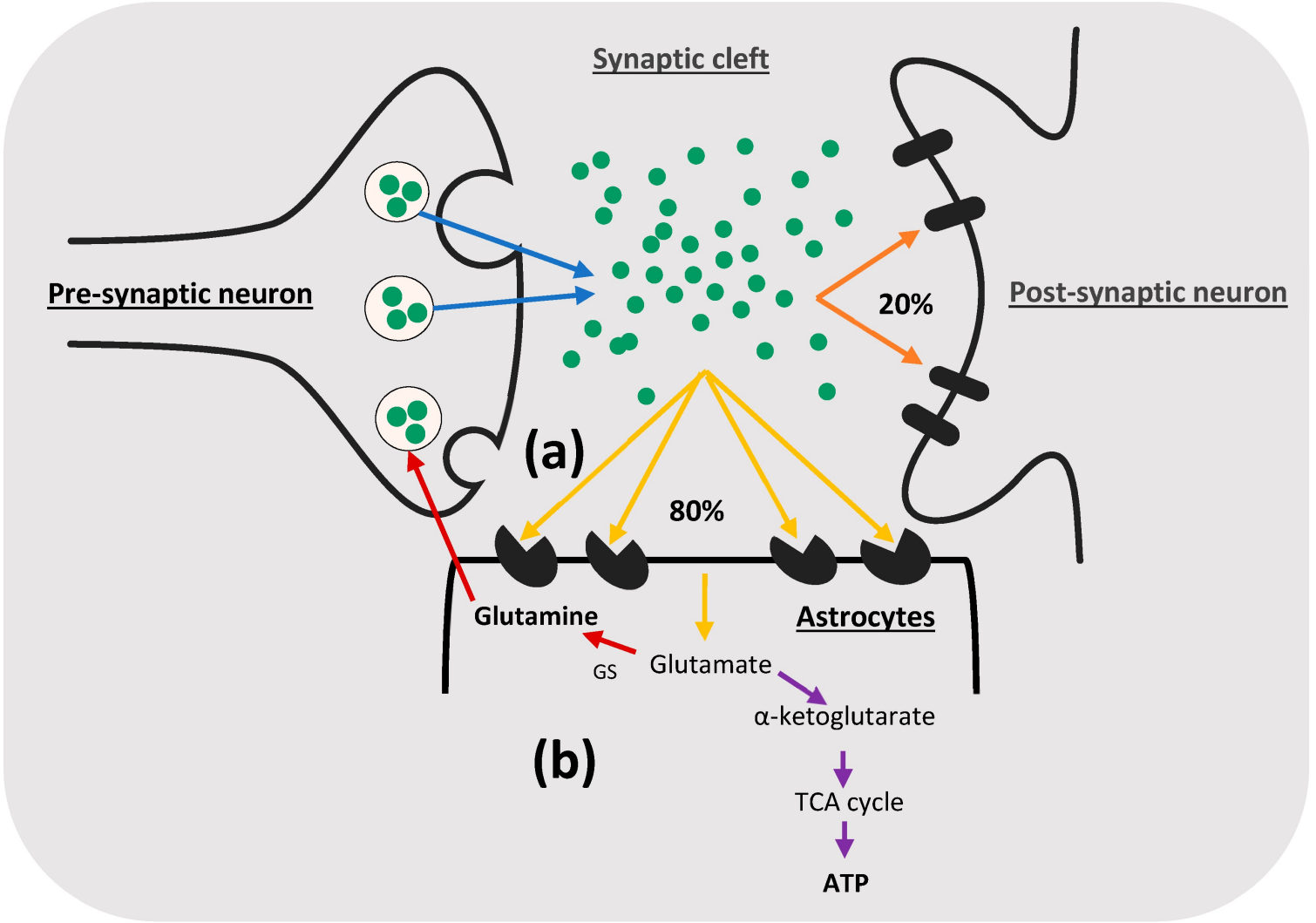
Astrocytes maintain glutamate homeostasis in the CNS



Regulation of extracellular glutamate concentration



Regulation of extracellular glutamate concentration



Glutamate Transporters

- ✓ The neuronal presynaptic reuptake pump (EAAT or excitatory amino acid transporter), Glutamate is transported across membranes of synapse by these Na^{++} dependent transporters.

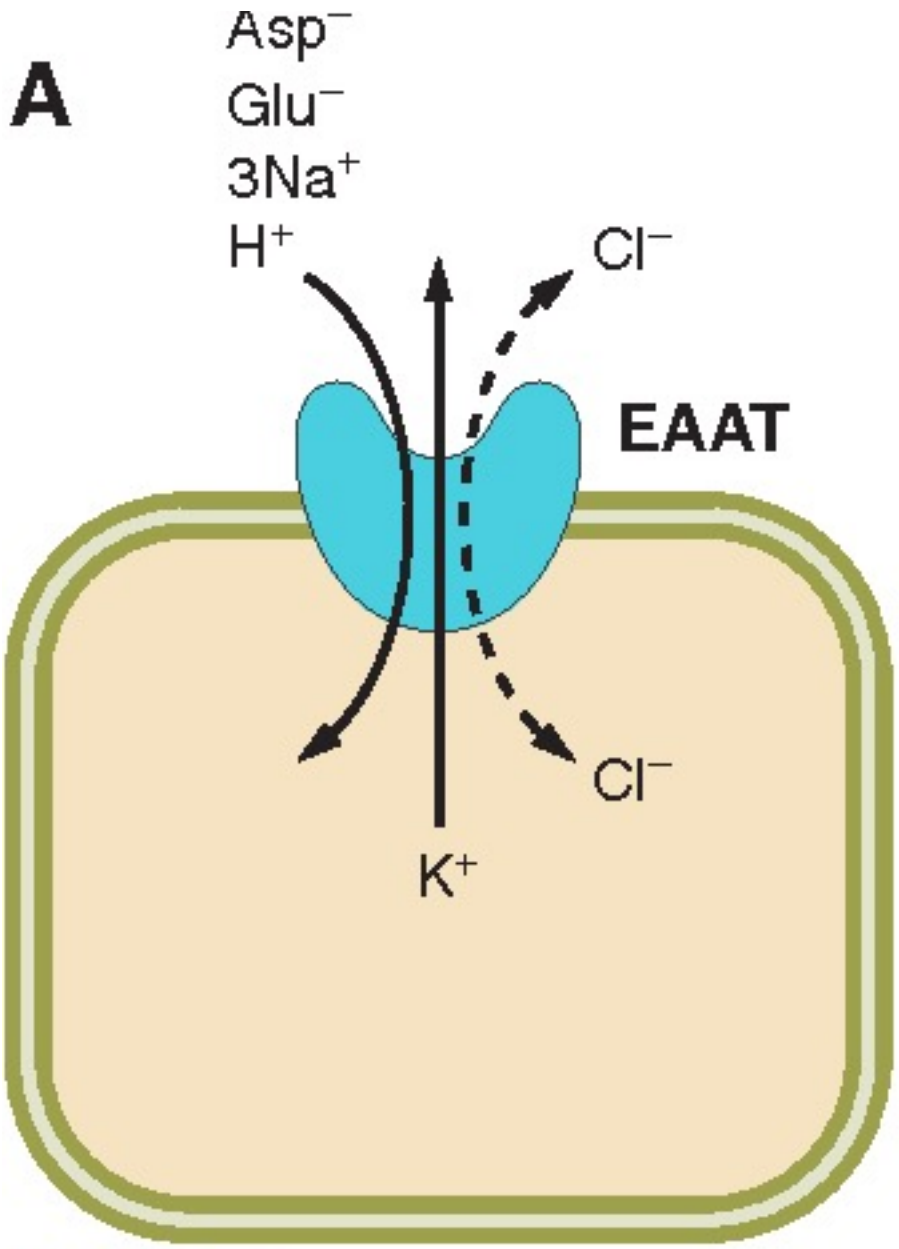
These are 5 types

- EAAT1 Astrocyte
- EAAT2 Astrocytes, Forebrain
- EAAT3 Upper motor neurons
- EAAT4 Cerebellar purkinje cells
- EAAT5 Retina

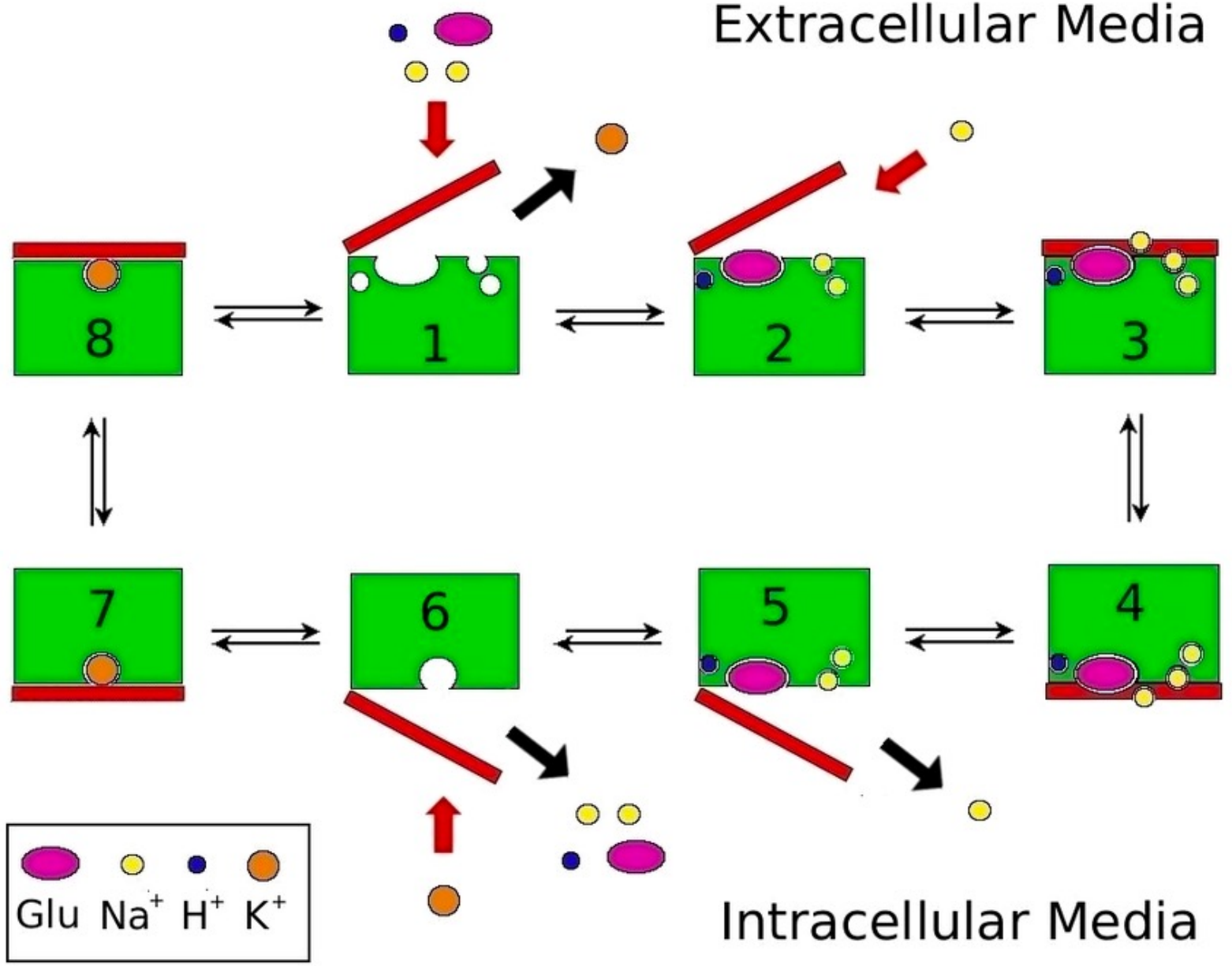
Of these EAAT1 & 2 are involved in the reuptake and release of glutamate during glutamine cycle

- ✓ The vesicular transporter for glutamate into synaptic vesicles (vGluT)

Excitatory Amino Acid Transporters



Excitatory Amino Acid Transporters



Elimination of Glu and GABA from the intersynaptic space by astrocytes

Glu = glutamate

GS = glutamine sintethetase

Gln = glutamine

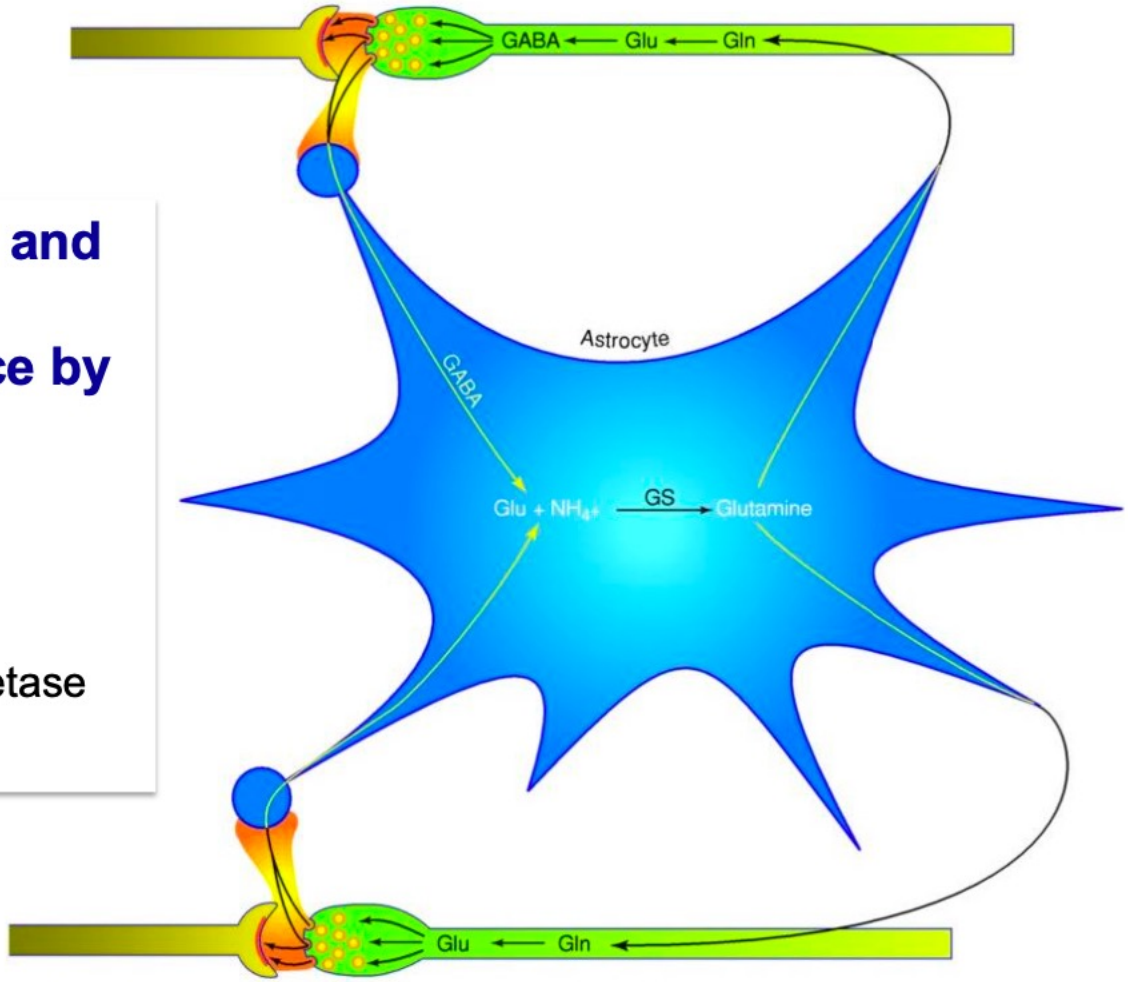
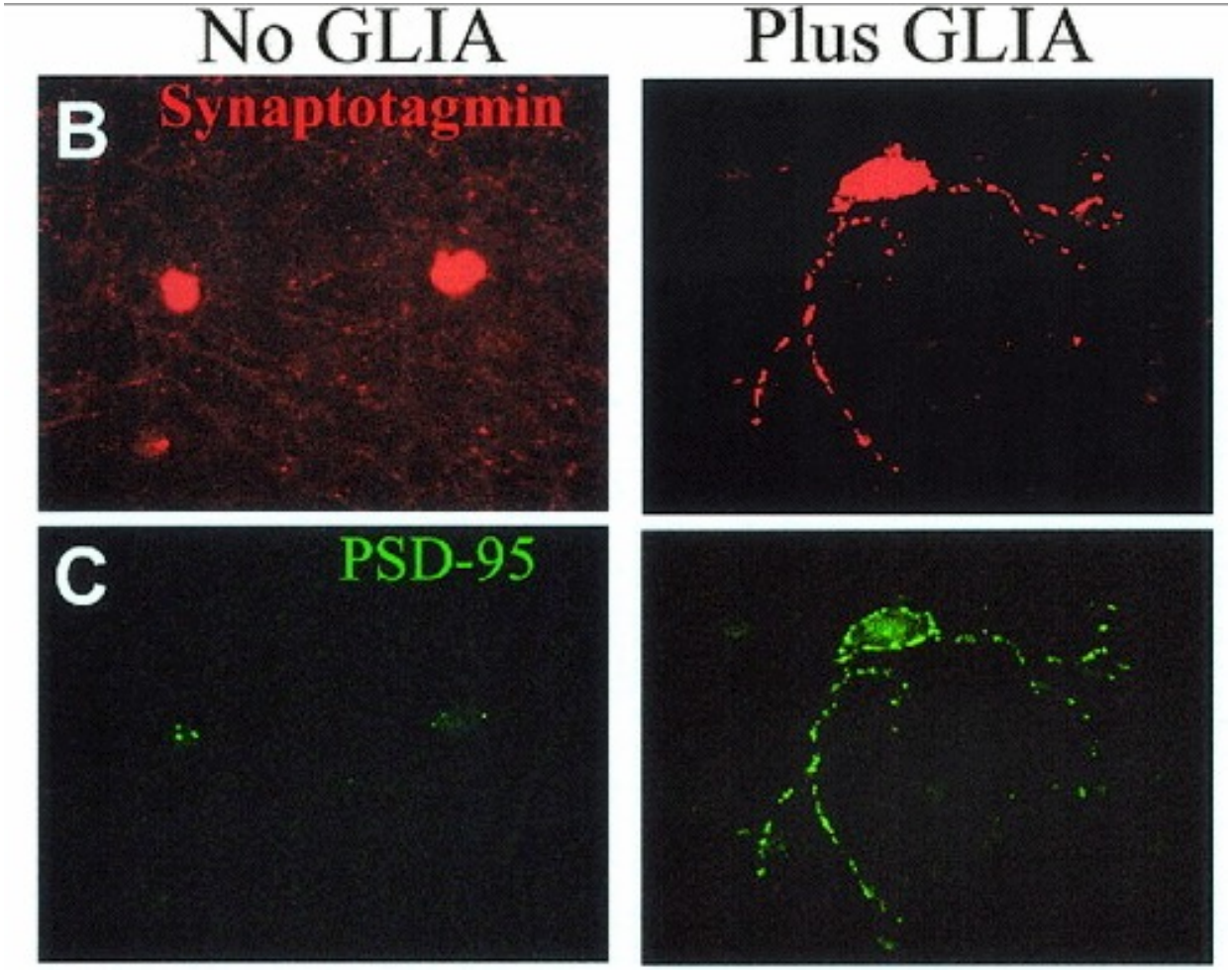


FIGURE 19 The glutamate–glutamine cycle is an example of a complex mechanism that involves an active coupling of neurotransmitter metabolism between neurons and astrocytes. The systems of exchange of glutamine, glutamate, GABA, and ammonia between neurons and astrocytes are highly integrated. The postulated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

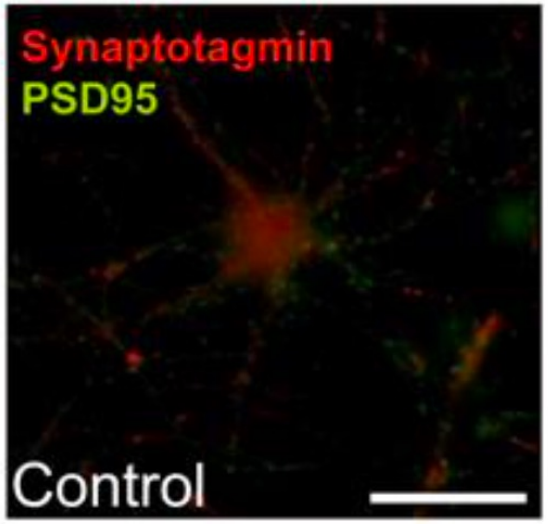
Astrocytes control synapse formation

Astrocytes control synapse formation

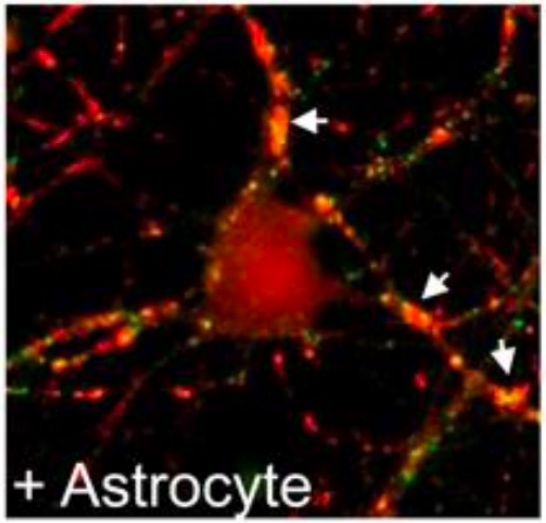


Control of Synapse Number by Glia
Erik M. Ullian, Stephanie K. Sapperstein,
Karen S. Christopherson, and Ben A. **Barres** *Science 2001*

Astrocytes play active roles in the formation of synapses



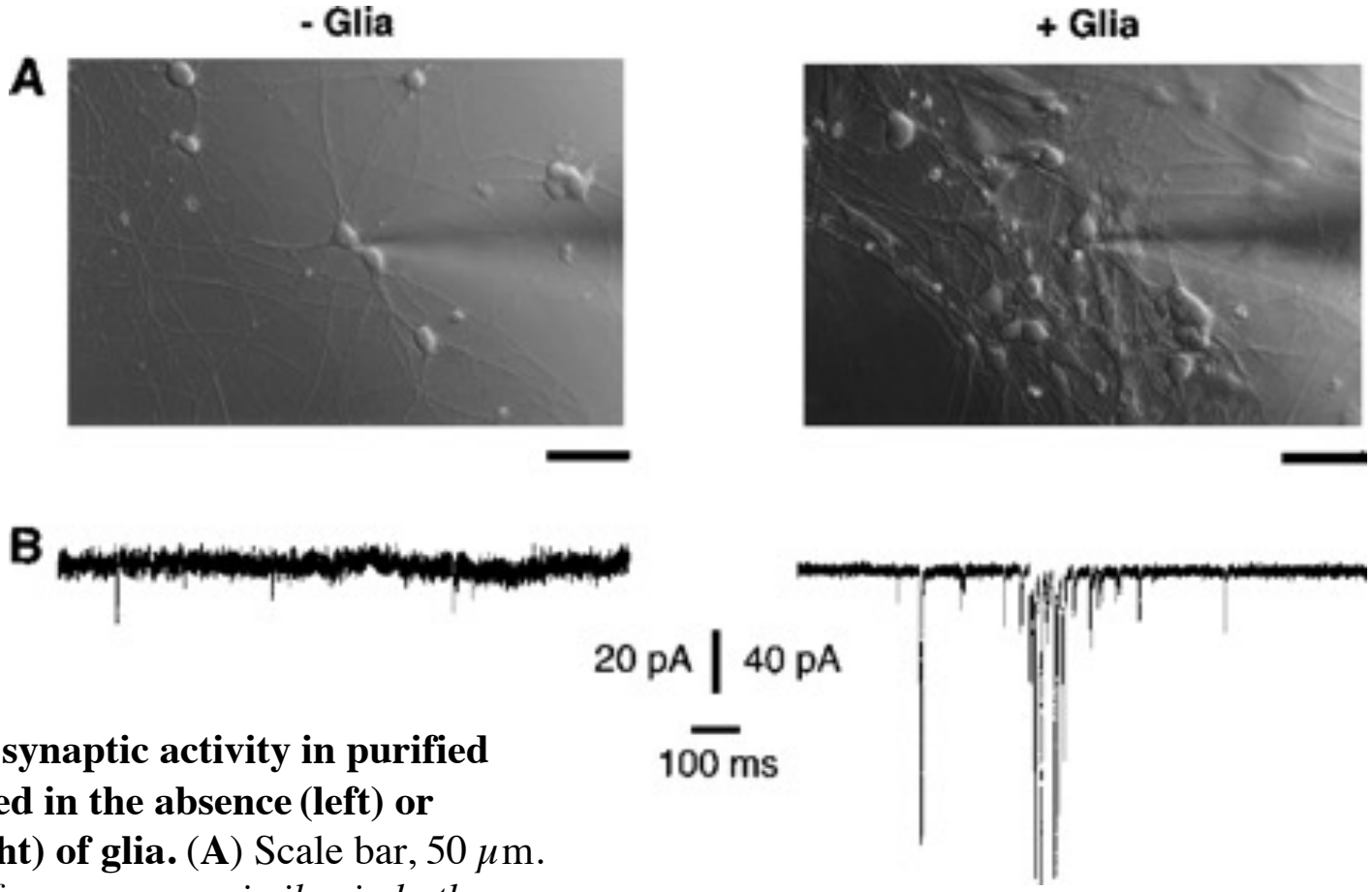
Purified retinal ganglion cells (RGCs) survive in culture, but show little spontaneous synapse activity and form few synapses



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

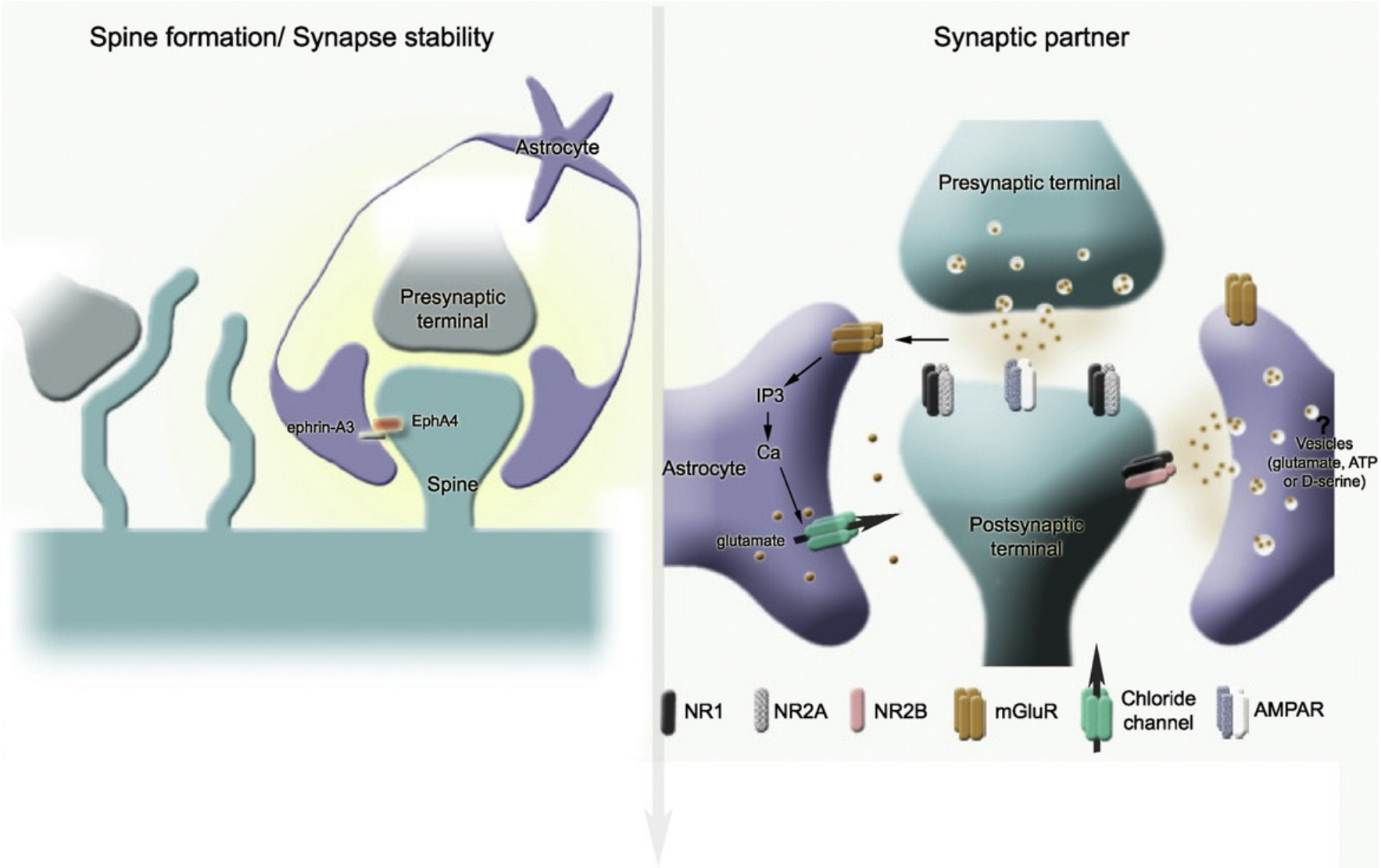
Astrocytes promote synaptogenesis



Spontaneous synaptic activity in purified RGCs cultured in the absence (left) or presence (right) of glia. (**A**) Scale bar, 50 μm. *The density of neurons was similar in both cultures*. (**B**) Whole-cell patch-clamp recordings of spontaneous EPSCs HP-70 mV

Science Vol. 277, 1684
Pfrieger and Barres

Astrocytes promote synaptogenesis

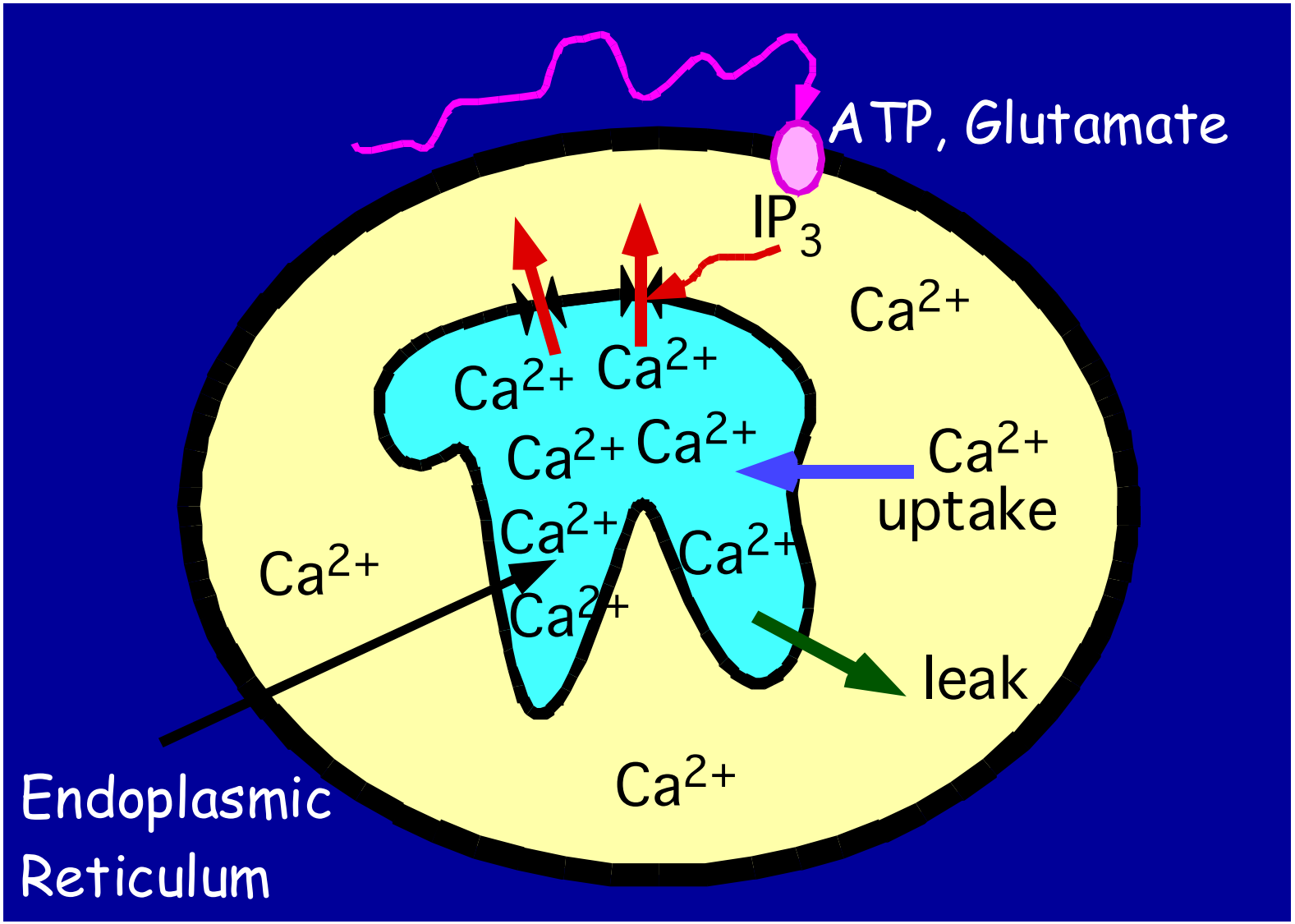


The new concept of Gliotransmission

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.

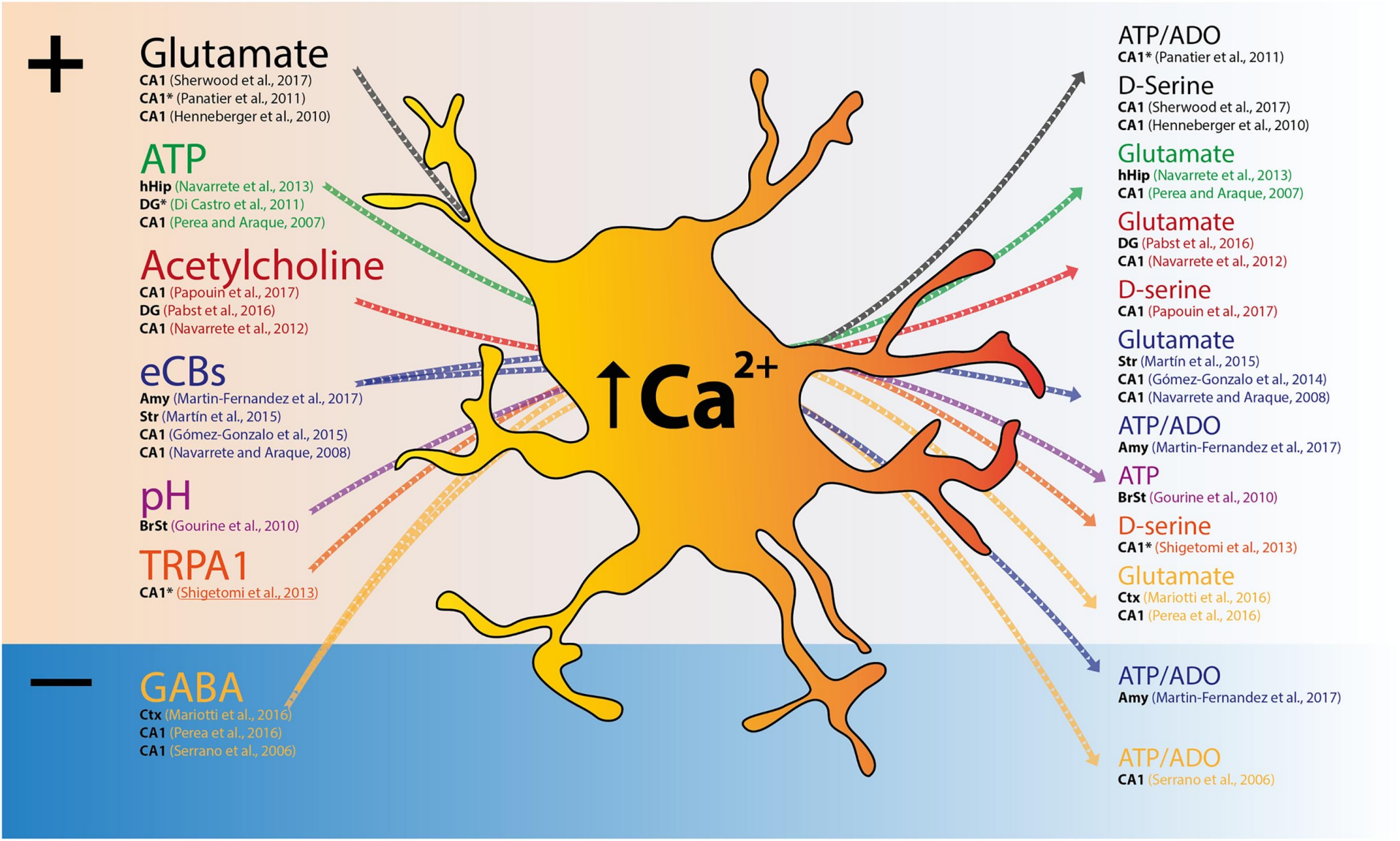
Two main forms of astrocyte excitation are well documented: one that is generated by chemical signals in neuronal circuits (neuron-dependent excitation) and one that occurs independently of neuronal input (spontaneous excitation).

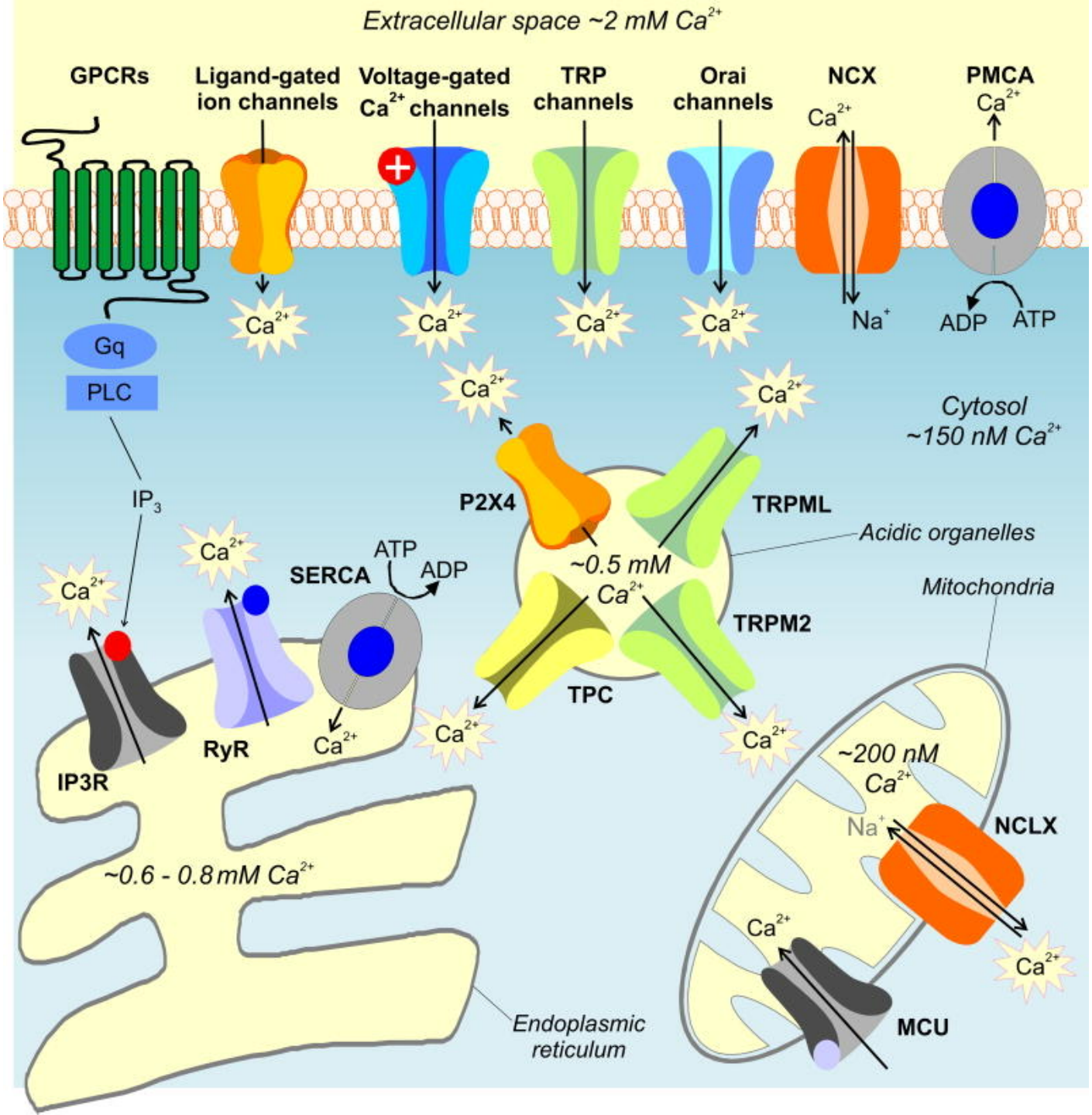
Astrocytes are Ca^{2+}- excitable



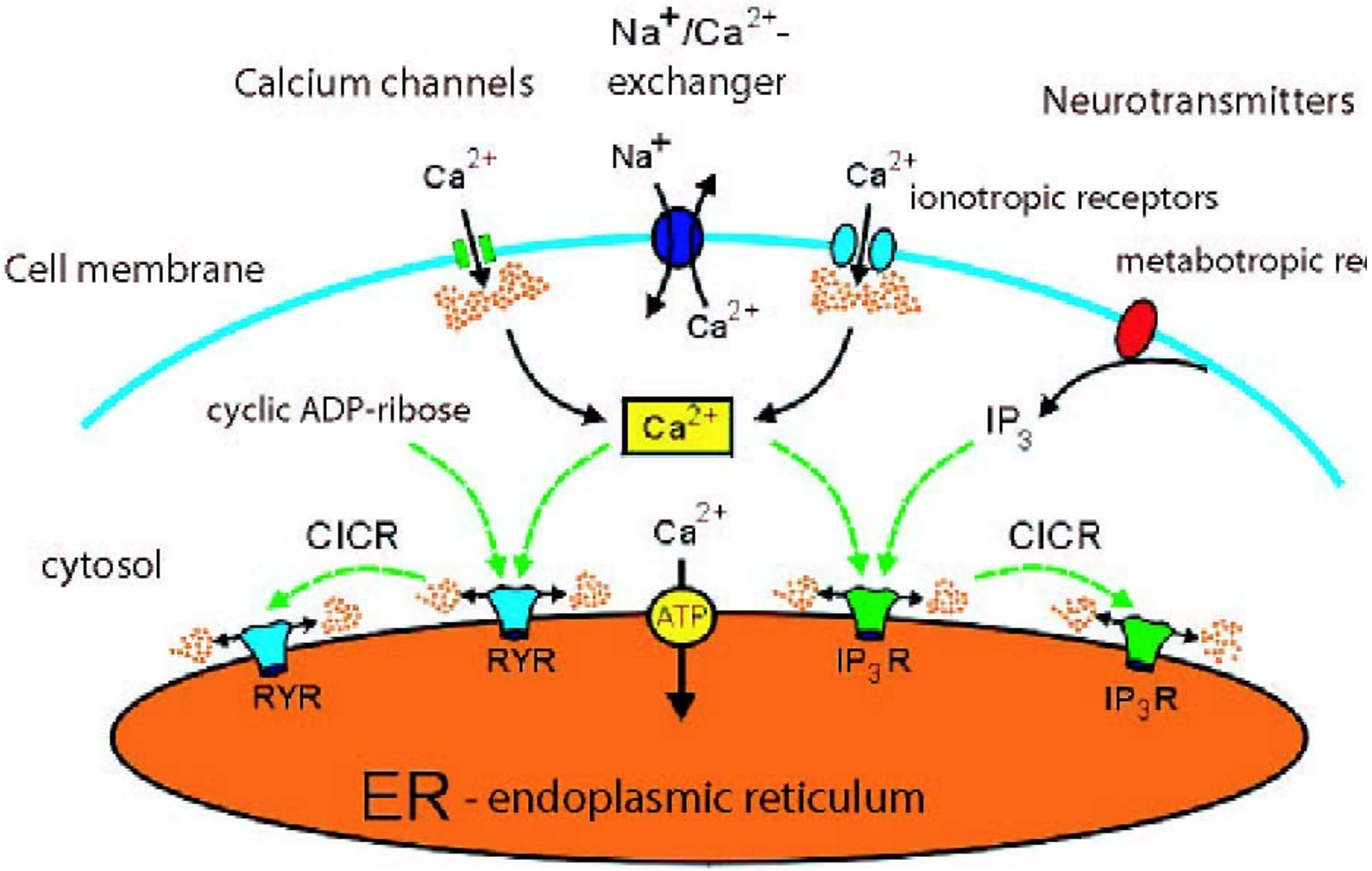
Cultured rat hippocampal astrocytes Cornell-Bell et al. Science, 247, 373 (1990)

Astrocytes are Ca^{2+}- excitable




Extracellular space ~2 mM Ca2+
GPCRs
Ligand-gated ion channels
Voltage-gated Ca2+ channels
TRP channels
Orai channels
NCX
PMCA
Ca2+
Na+
ADP
ATP
Gq
PLC
IP3
Cytosol ~150 nM Ca2+
P2X4
TRPML
Acidic organelles
~0.5 mM Ca2+
TRPM2
TPC
SERCA
ATP
ADP
RyR
IP3R
Mitochondria
~200 nM Ca2+
NCLX
MCU
~0.6 - 0.8 mM Ca2+
Endoplasmic reticulum

Intracellular Calcium handling



Positive feedback via CICR - excitable dynamics

Calcium Induced Calcium Release

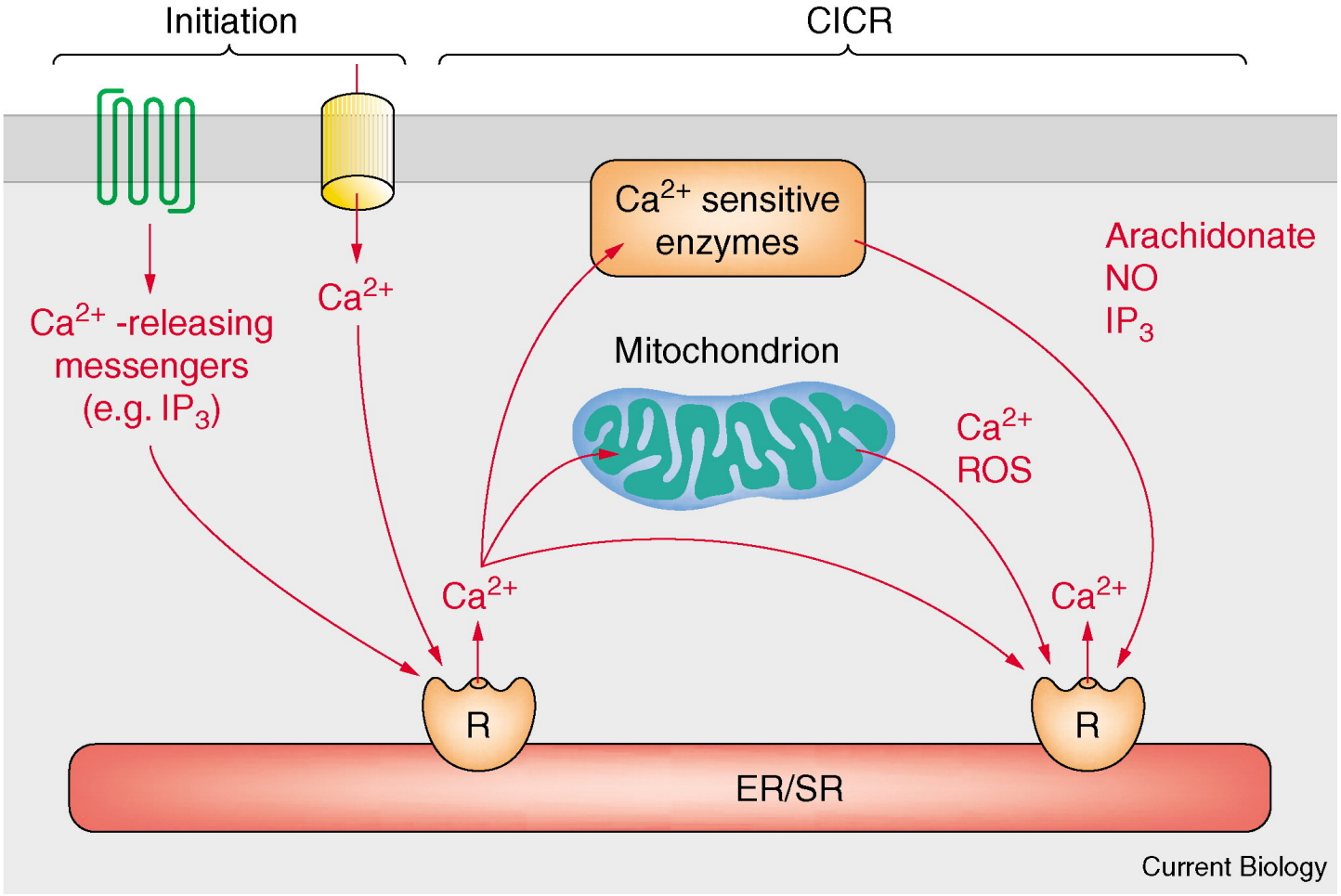
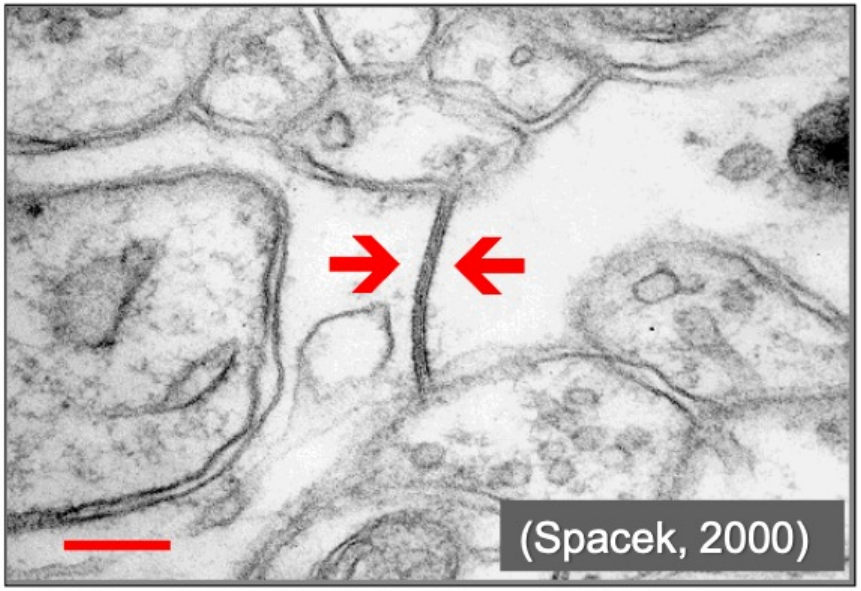


Figure 1.
This cartoon depicts how CICR mechanisms amplify calcium signals. Starting from the left-hand side, the figure illustrates the initiation of a calcium signal via an intracellular channel ('R''). This leads to direct release of calcium from neighbouring channels or production of calcium-releasing messengers.

Astrocyte network

Astrocyte-astrocyte communication



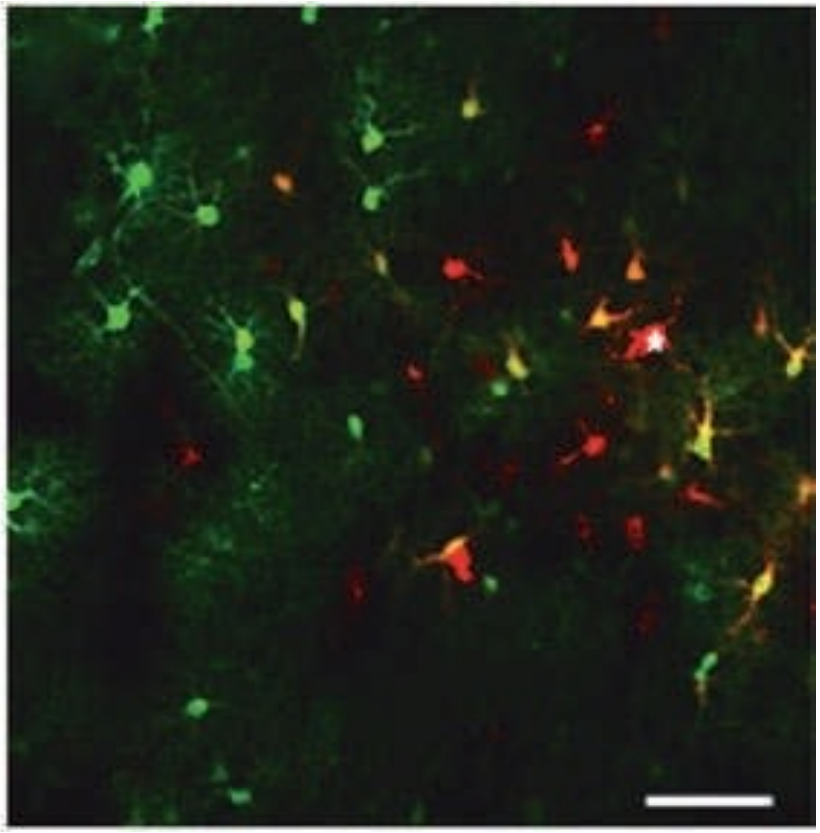
Cells are linked together by gap junctions

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.

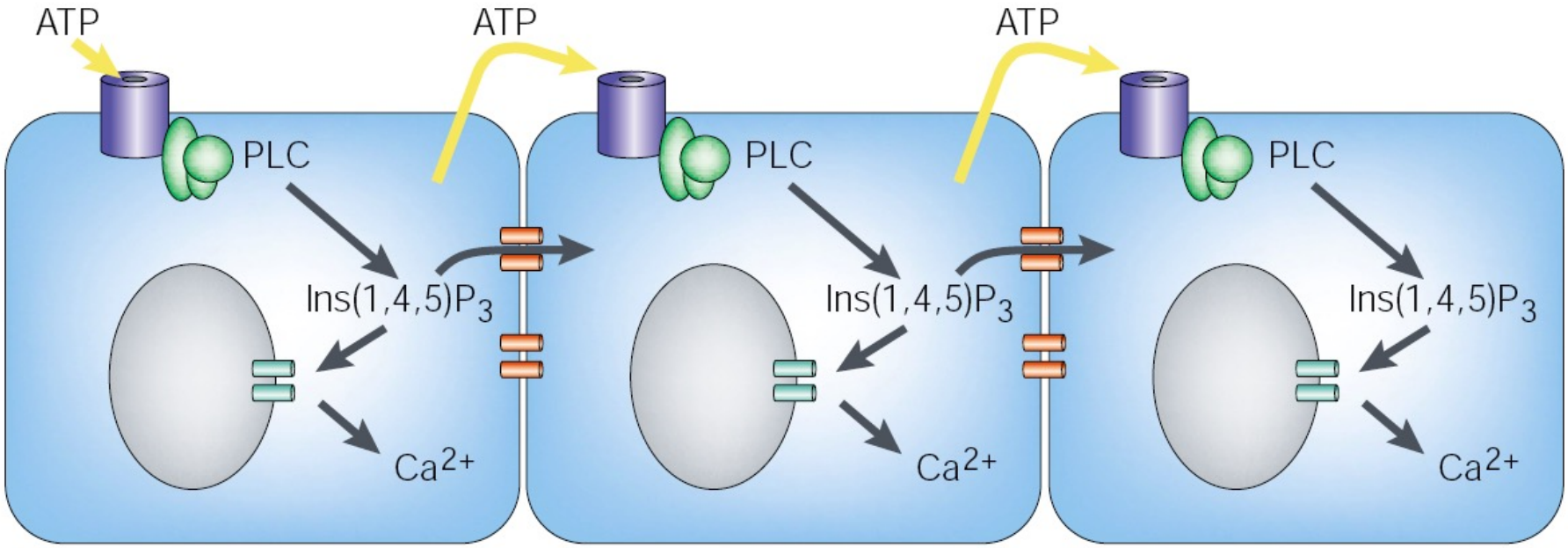
Astrocyte network

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



Intercellular Ca^{2+} Waves

(Cornell-Bell et al., M. Sanderson, A. Charles)



Speed: ~20μm/s

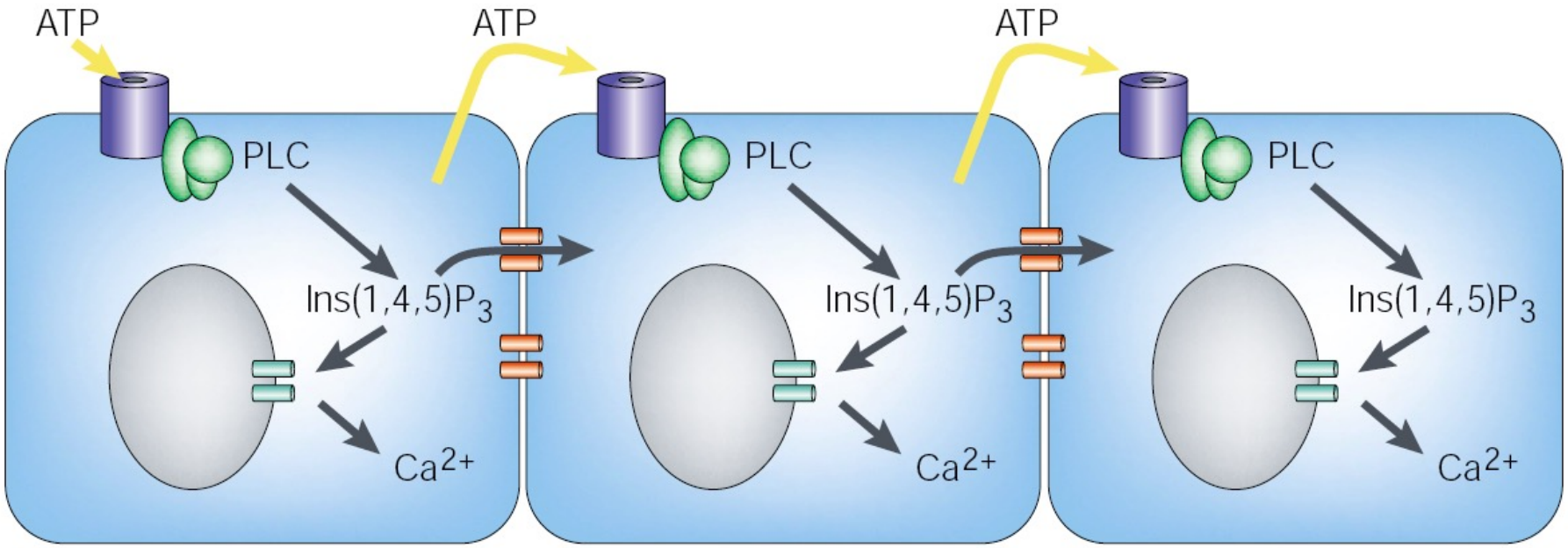
Range: a few hundred μm

Time scale: seconds to minutes

Intercellular Ca^{2+} Waves

(Cornell-Bell et al., M. Sanderson, A. Charles)

- Direct intercellular diffusion of IP_3
- Regenerative release of diffusible extracellula messenger
- Diffusion
- Involvement of neurons, oligodendrocytes and microglia



The new concept of Gliotransmission

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).

Neurotransmitter/Gliotransmitter release from astrocytes

Non vescicular release

- Reverse transport
- Volume activated anion channels
- Hemichannels

Vescicular release

- Astrocytes possess all the main proteins involved in exocytosis, vescicular glutamate transporter (VGLUT)
- Source of Ca^{2+}
- Slower than in neurones

Gliotransmission: the release of various chemical transmitters from astrocytes

Transmitter	Target receptor	Actions
Glutamate	NMDA	Synchronous depolarization
	Kainate	Increases miniature postsynaptic current frequency
	mGlu	Increases probability of release and AMPA-receptor-dependent miniature postsynaptic current frequency
ATP	P2X	Insertion of AMPA receptors into postsynaptic site
	P2Y	Paracrine actions in astrocytic Ca2+ waves in cultures
	A1	When degraded to adenosine results in suppression
d-Serine	NMDA	Increases NMDA-dependent synaptic transmission, important for the induction of LTP in many brain regions

Astrocyte - Neuron Communication

Astrocyte - Neuron Communication

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

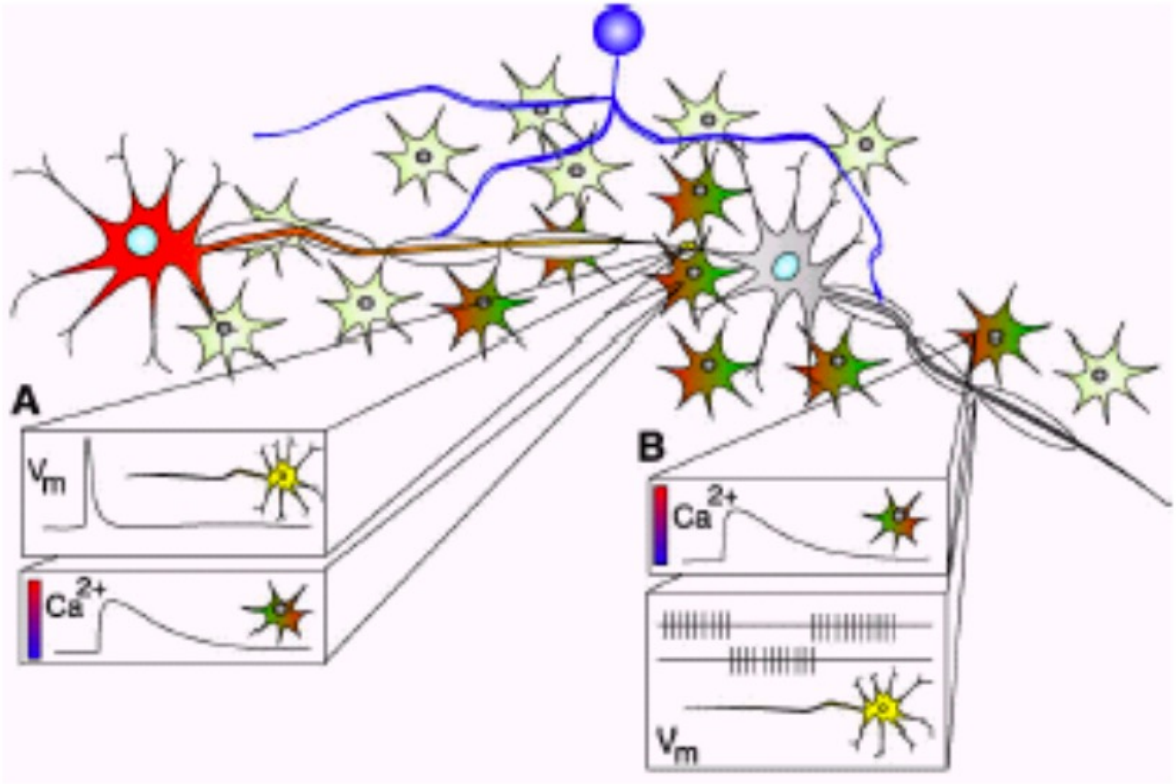
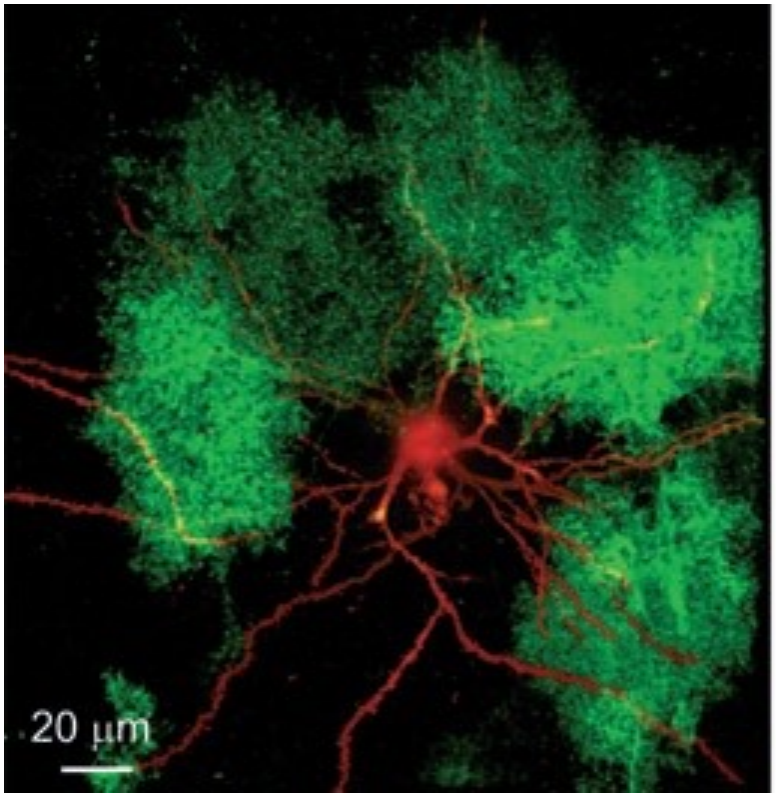


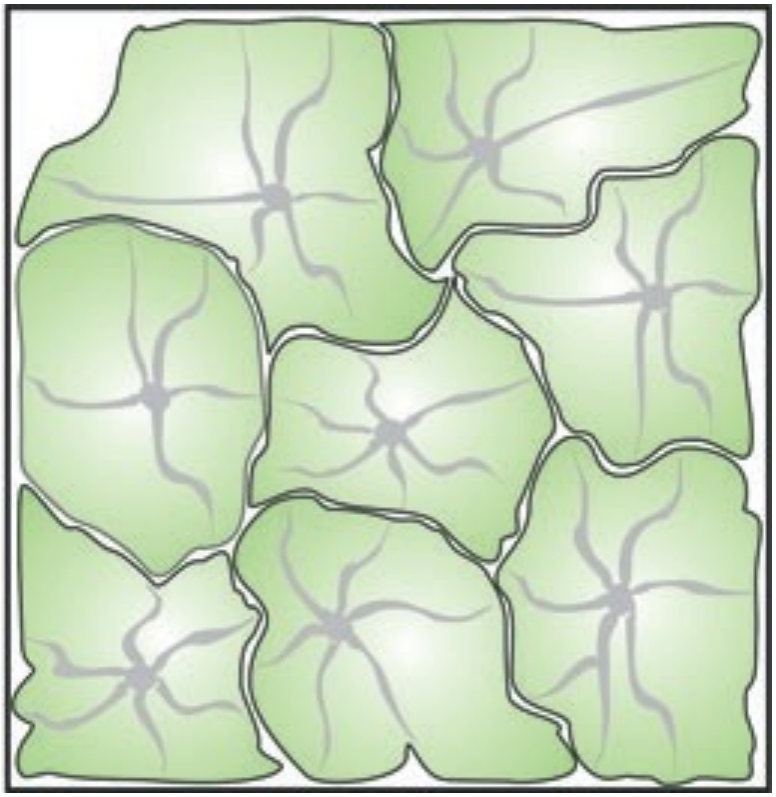
Fig. 2. Calcium imaging reveals communication between neurons and glia. (A) Molecules released during synaptic transmission bind receptors on glia that cause increases in intracellular Ca^{2+} (rainbow colored cells), which are propagated as waves through glial networks. (B) Increases or decreases in 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

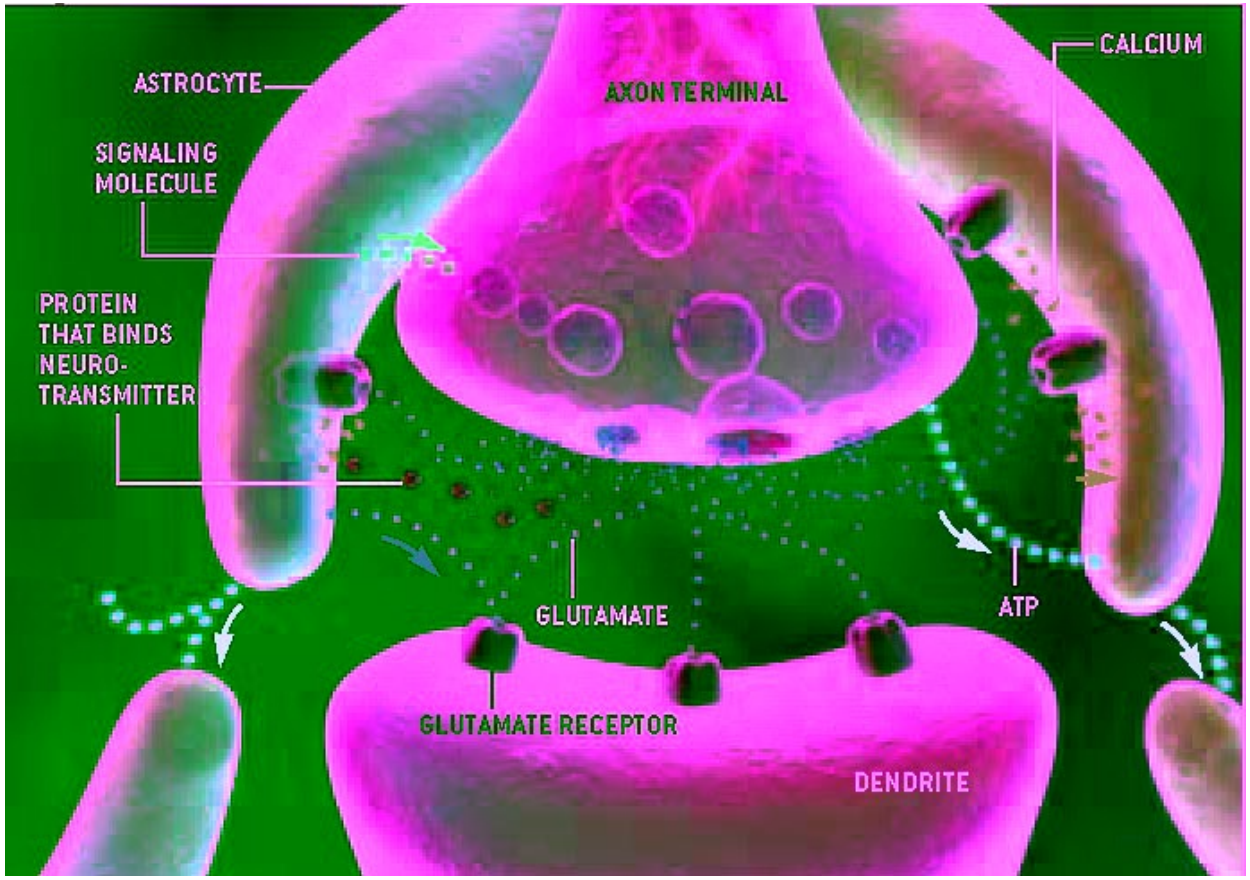
Astrocyte - Neuron Communication

- Astrocytes enwrap synaptic structures
- Specialized appendages
- Indipendent compartments
- Tripartite synapse
- Neuronal-glial synapses



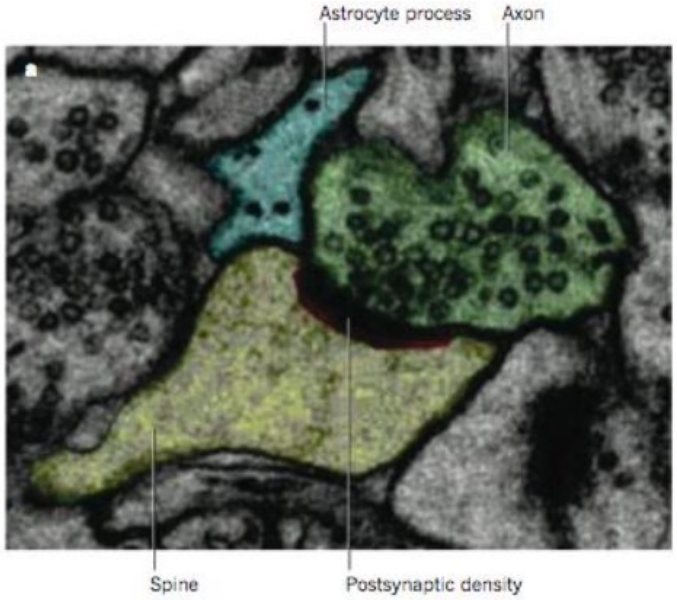


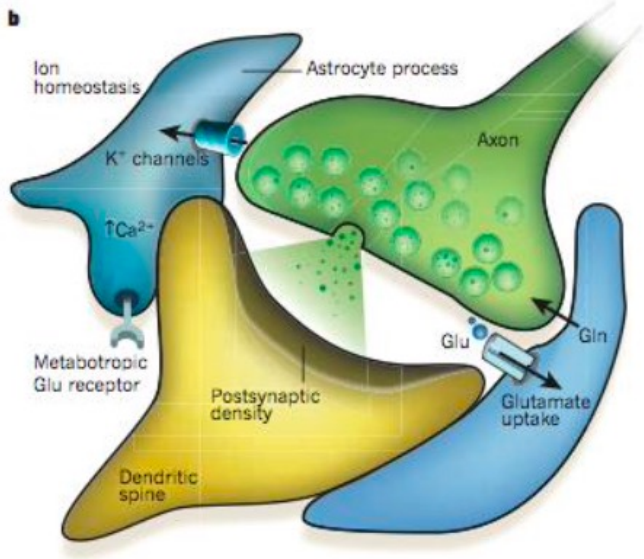
The tripartite synapse



From Fields, Scientific American

The tripartite synapse

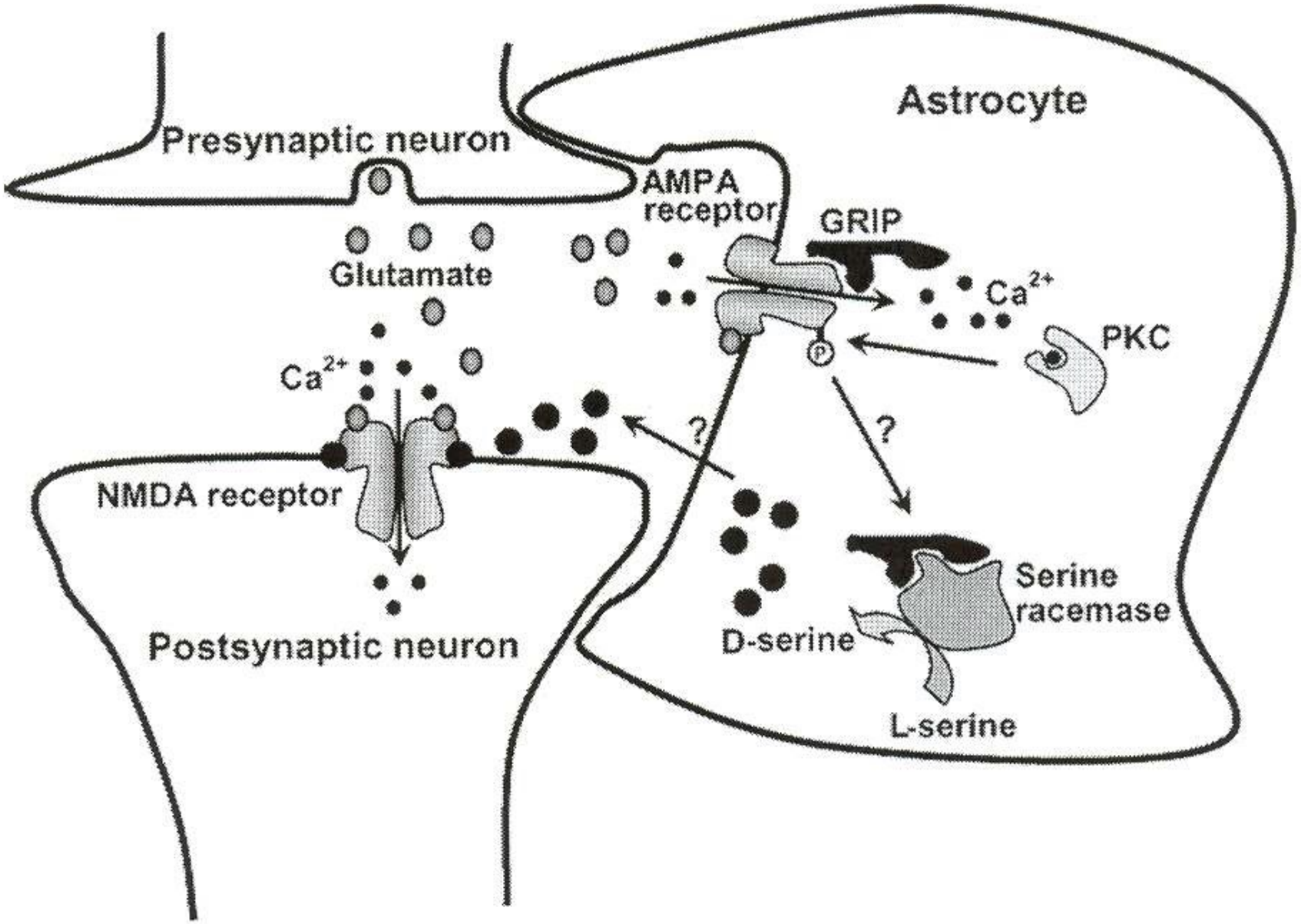




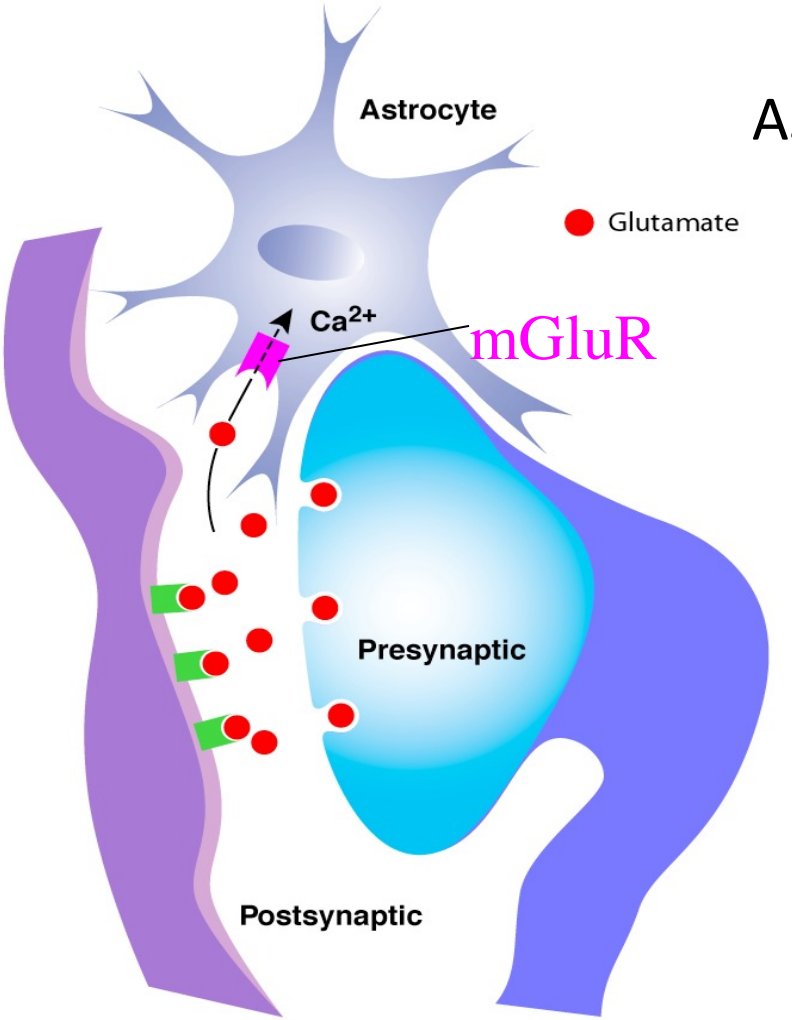
Eroglu and Barres, 2010, Nature
doi:10.1038/nature09612)

- 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

The tripartite synapse

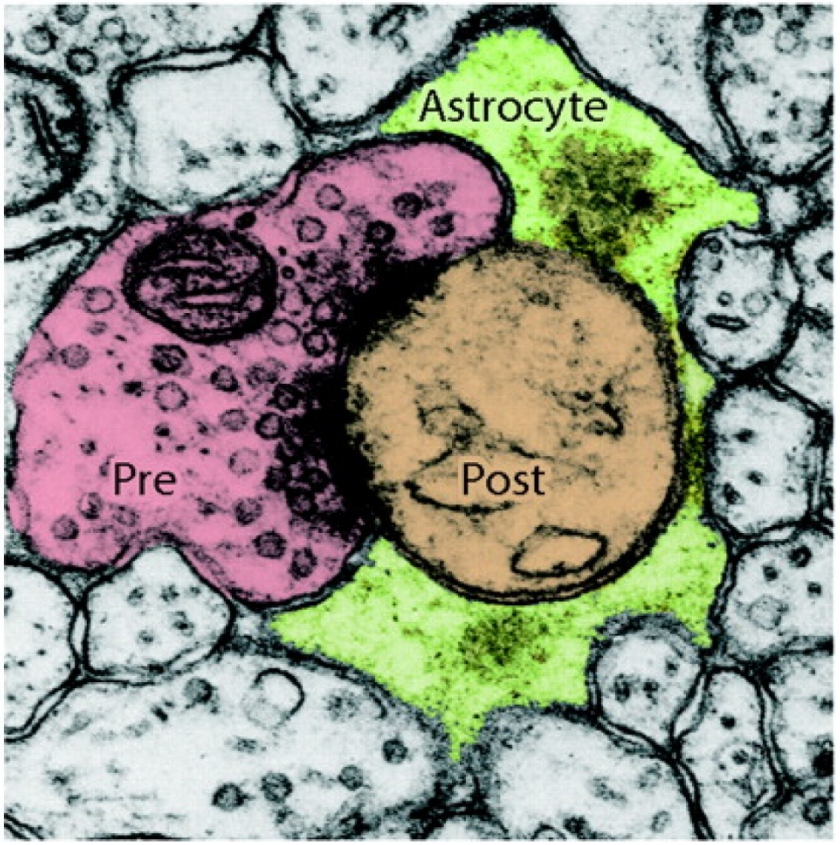


Neurons talk to Astrocytes



Tripartite Synapse
(Araque et al. Trends Neurosci 22 (1999))

Astrocyte processes enwrap synapse



Fellin et al., Physiology 21, 208 (2006)

Astrocyte sense neuronal activity

Stimulation of neuronal fibres induce Ca^{2+} signals in astrocytes
Isolated compartments
Plasticity (frequency)

Bergmann glia at parallel fibers-Purkinje neurons synapses

Gliotransmission: the release of various chemical transmitters from astrocytes

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 synaptic-like vescicles

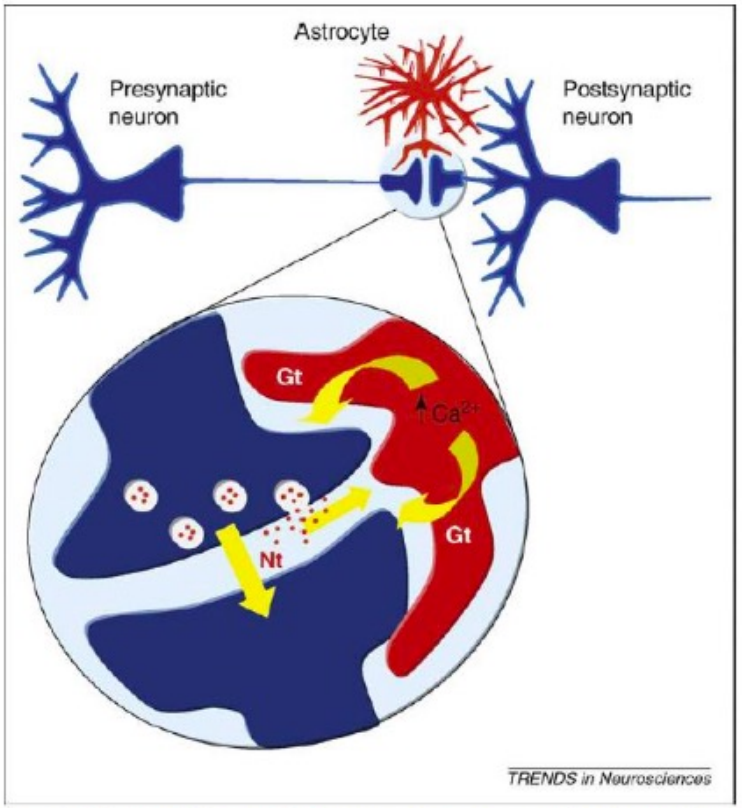
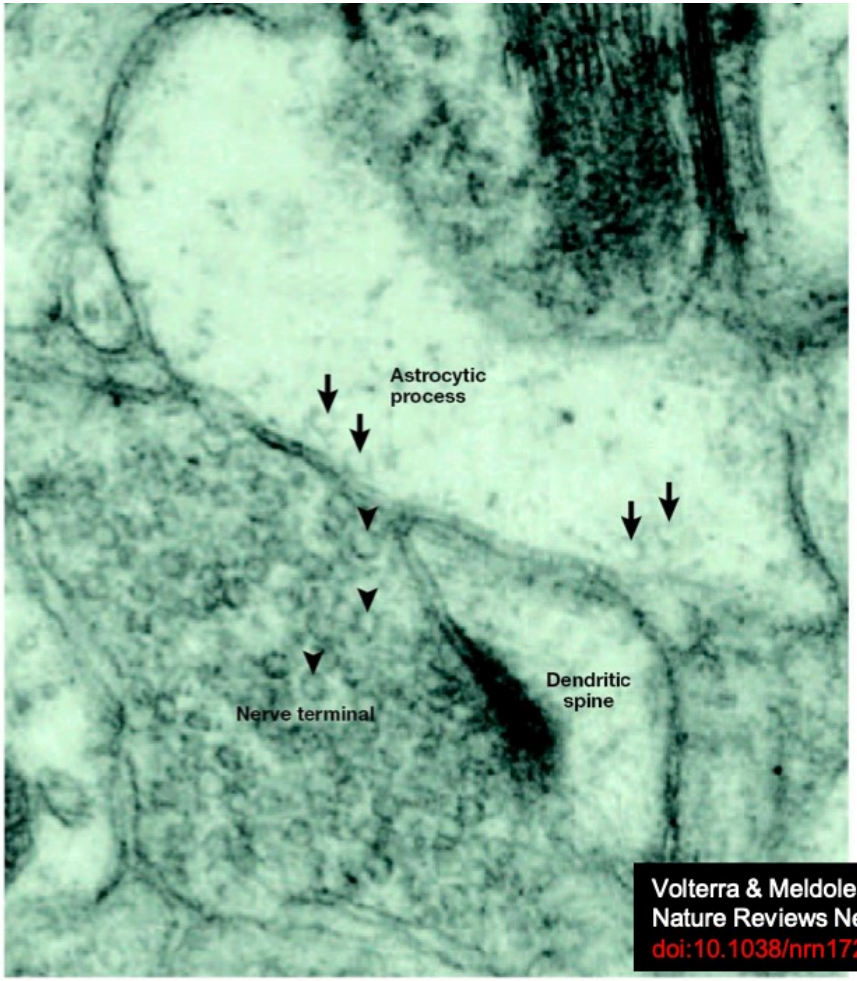


Figure 2. Scheme of the tripartite synapse. Cartoon representing the transfer of information between neuronal elements and astrocyte at the tripartite synapse. Astrocytes respond with Ca^{2+} elevations to neurotransmitters (Nt) released during synaptic activity and, in turn, control neuronal excitability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



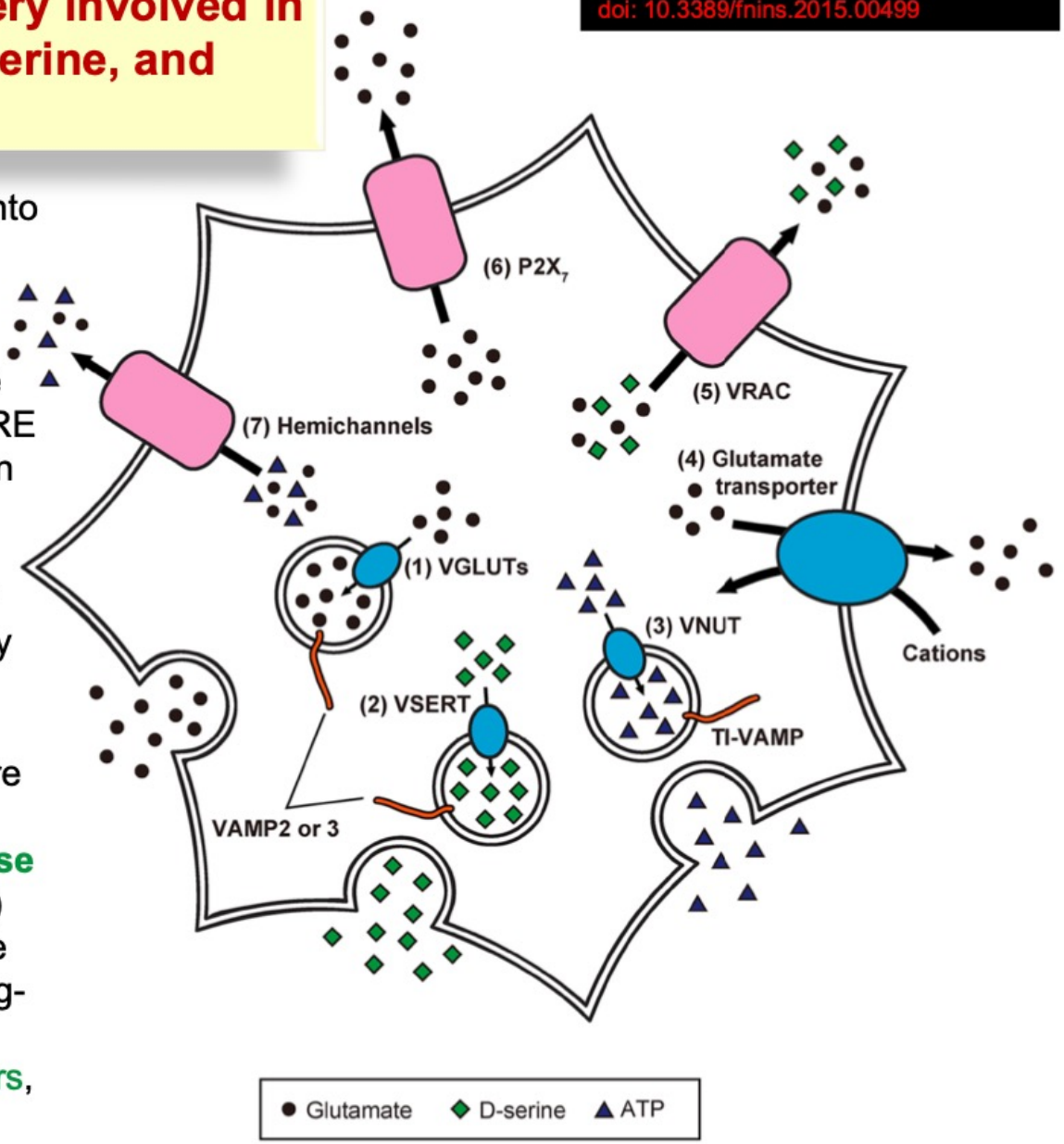
Volterra & Meldolesi, 2005
Nature Reviews Neurosci
doi:10.1038/nrn1722

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).

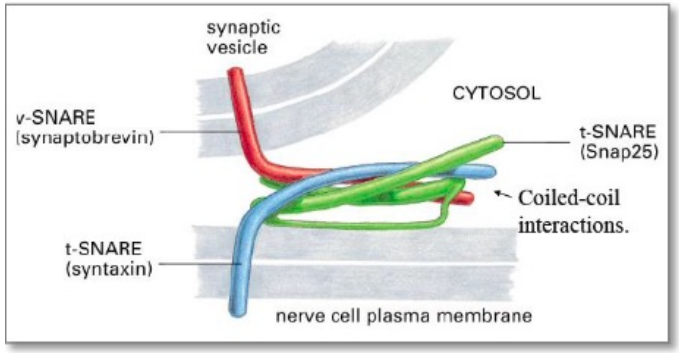
Precise intracellular machinery involved in the release of glutamate, D-serine, and ATP from astrocytes

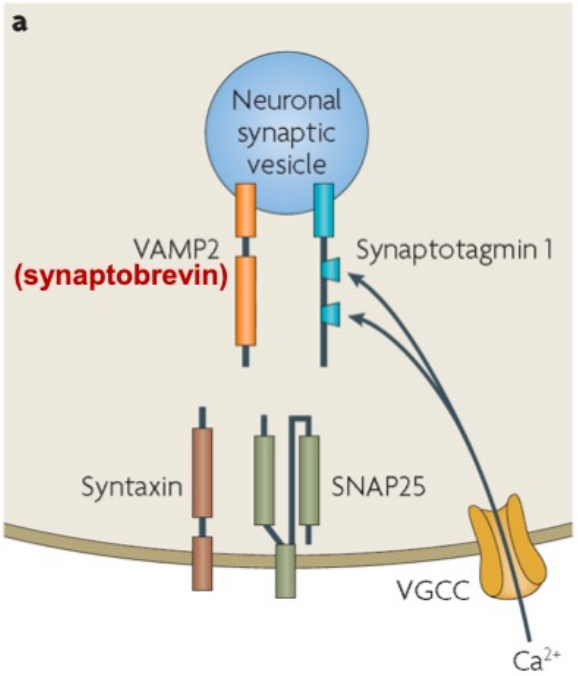
Harada et al., 2016, Frontiers in Neurosci.
doi: 10.3389/fnins.2015.00499

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





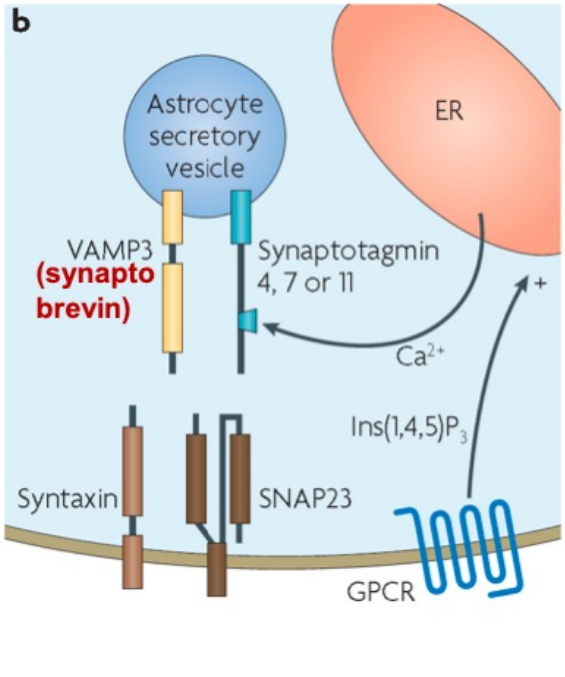
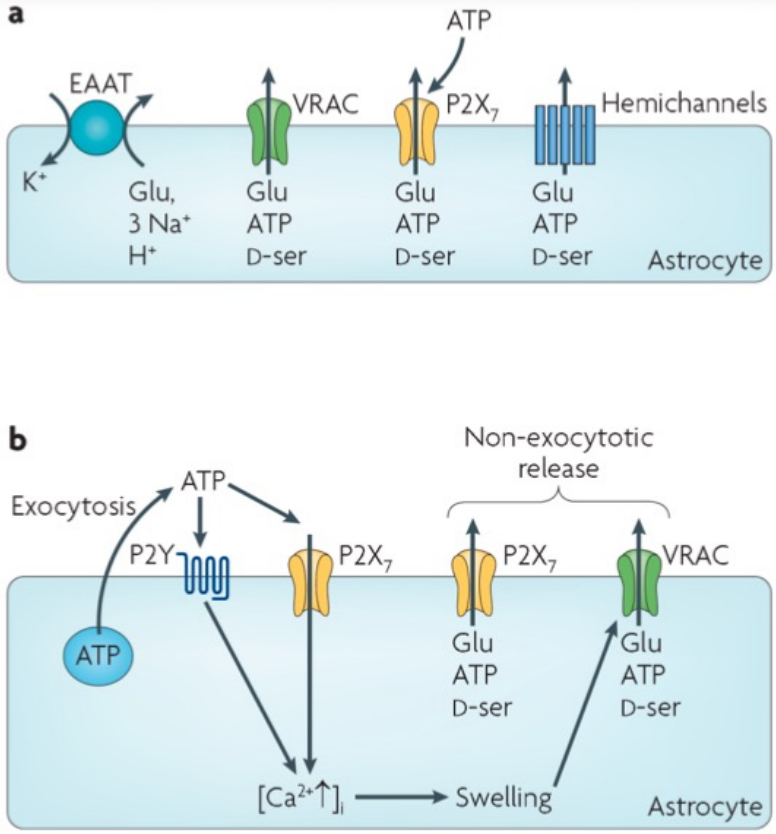
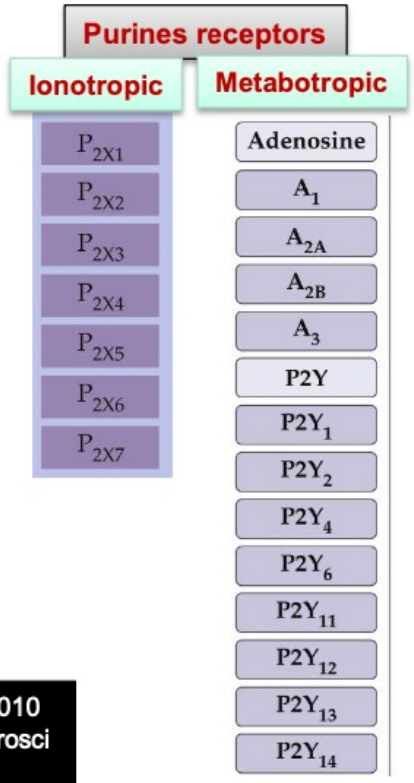


Figure 3 | **Proteins proposed to mediate exocytosis from neurons and astrocytes.** **a** | For the formation of a functional SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) complex that mediates vesicle fusion, syntaxin and synaptosomal-associated protein 25 (SNAP25) 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

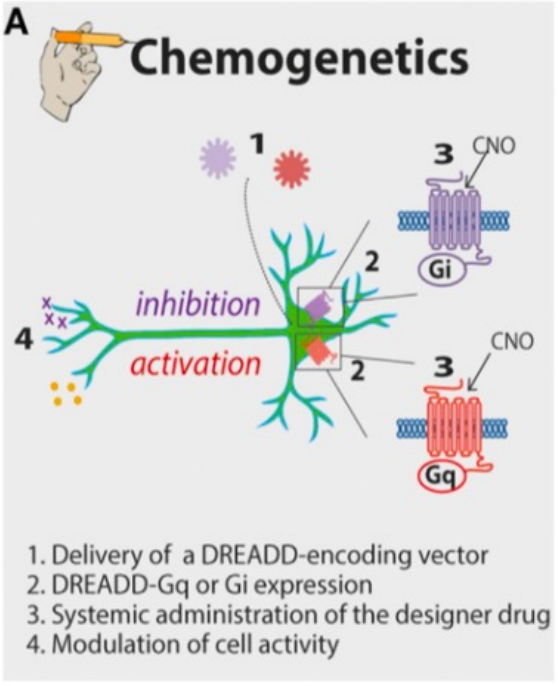
a | **Non-exocytotic transmitter release** can occur by reversal of plasma membrane glutamate (Glu) transporters (excitatory amino-acid transporters (EAATs)), or (for glutamate, ATP and d-serine (d-ser)) by efflux through volume-regulated anion channels (VRACs), ATP-gated P2X purinoceptor 7 (P2X7) receptor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

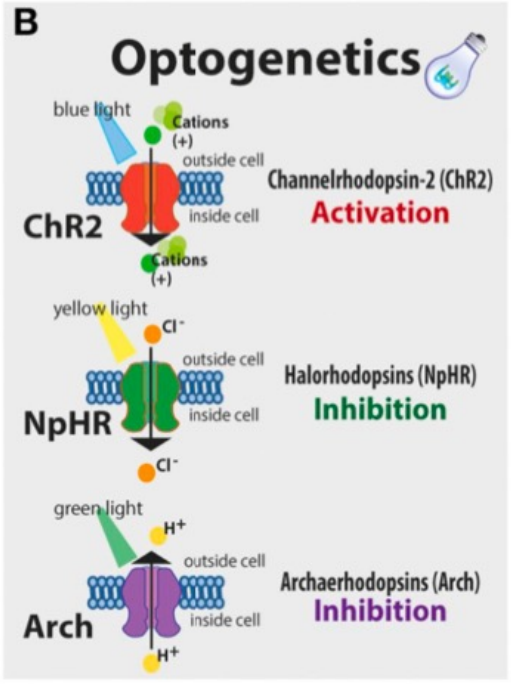
Optogenetic and Chemogenetic Approaches for Studying Astrocytes and Gliotransmitters

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.

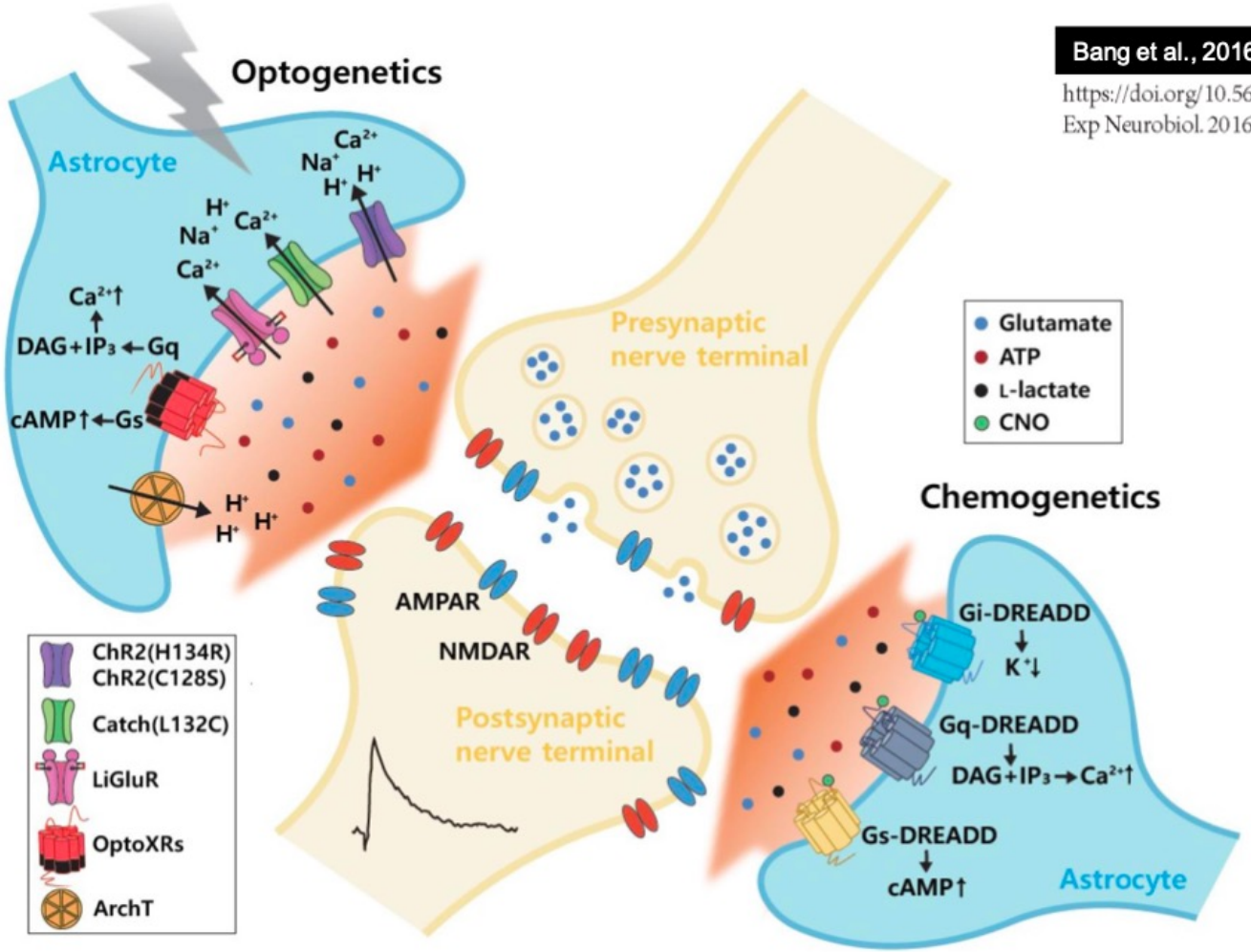
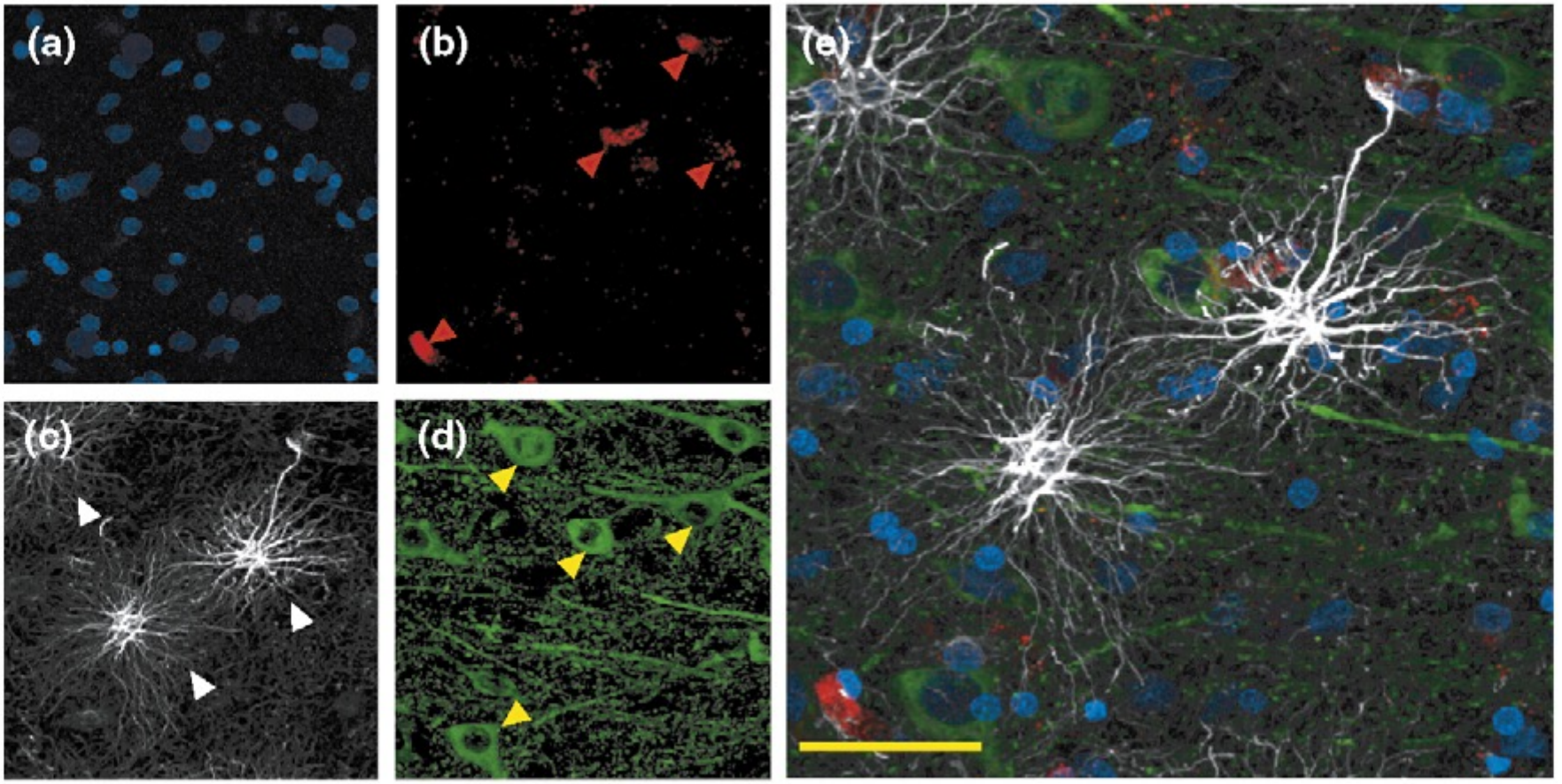


Fig. 1. Optogenetic and chemogenetic stimulation of astrocytes. A variety of genetically encoded effector molecules for optogenetics (left) and chemogenetics (right) have been employed to manipulate intracellular ionic concentrations (H^+, Na^+, Ca^{2+}, K^+) and signaling cascades (Gq, Gs, DAG, IP_3, 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.

Astrocytic domains

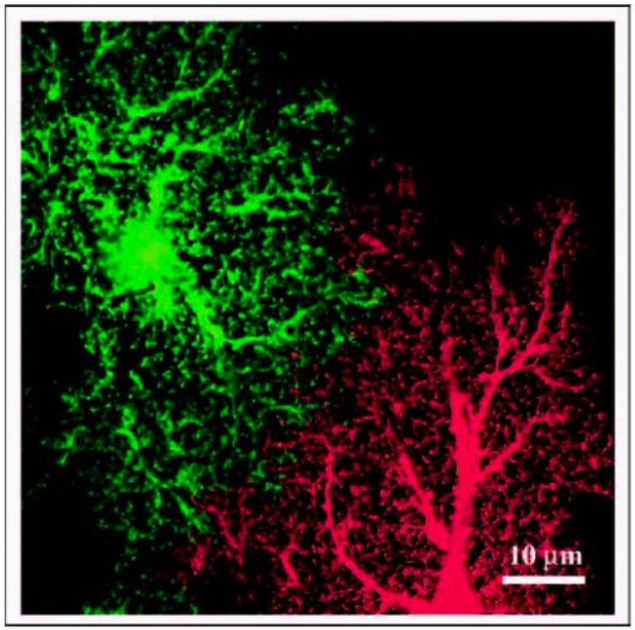


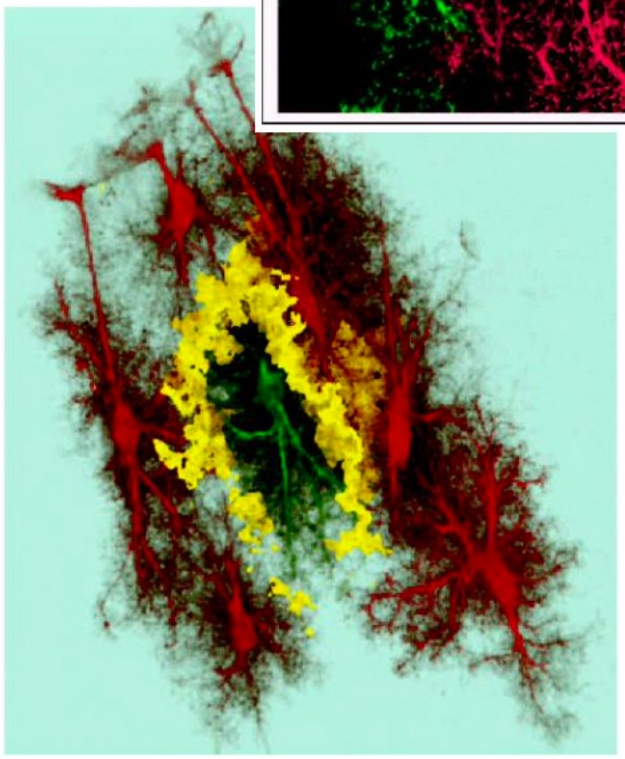
Astrocytic domains

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

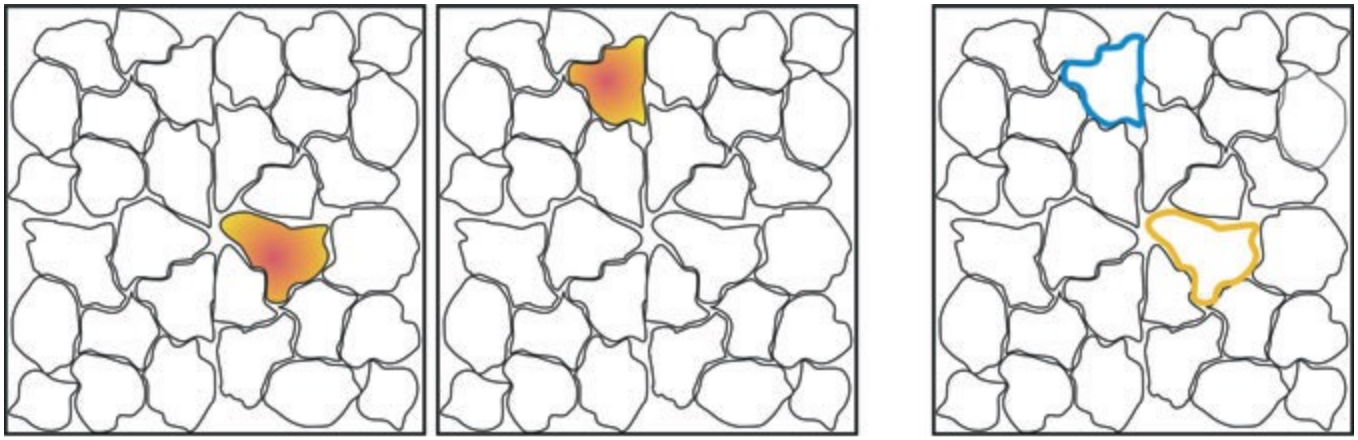




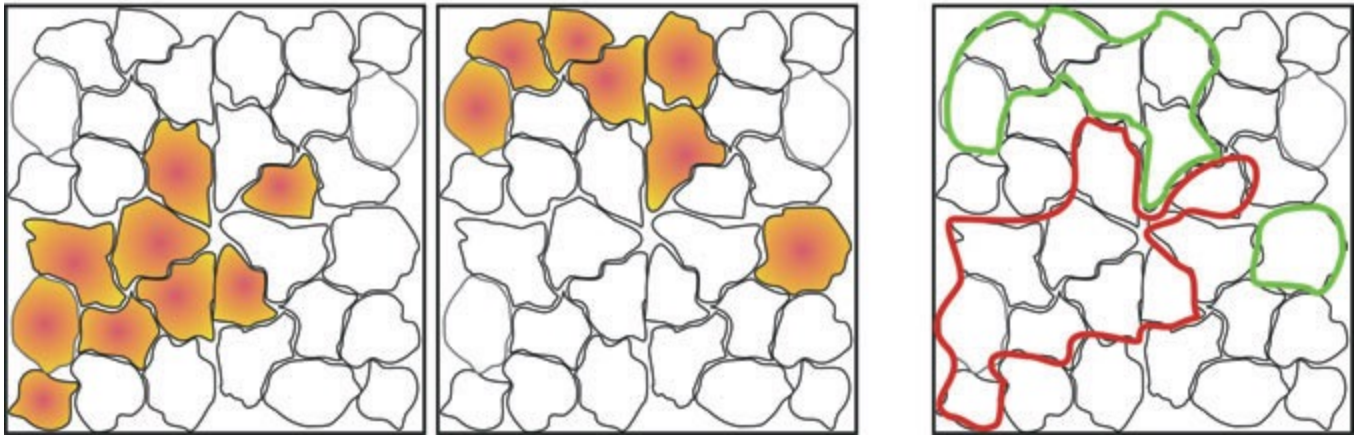
Astrocytic domains

Astrocytic Ca2+ signaling in vivo is organized in functional domains.

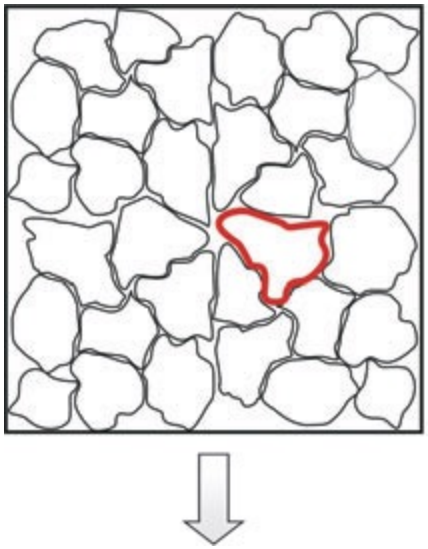
spontaneous

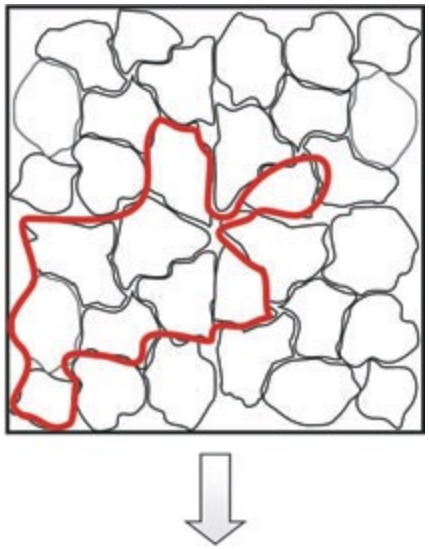


Activity-evoked



Astrocytic domains





Local neuronal
Modulation
Single domain

Widespread neuronal
Modulation
Multiple domains

Astrocytic domains

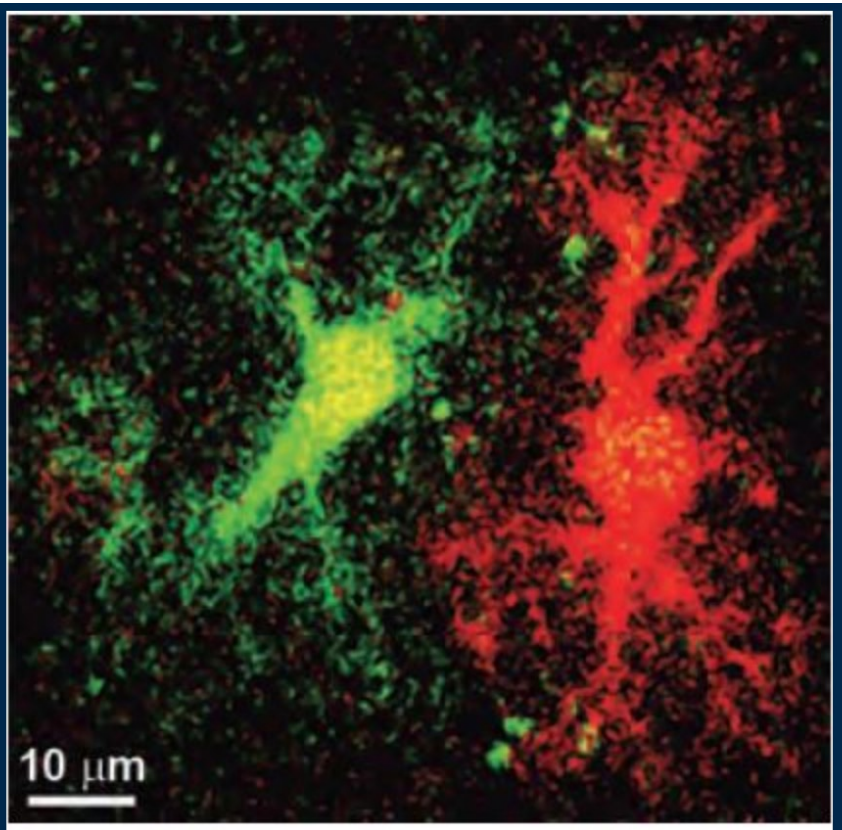


Figure 1. Astrocytes occupy distinct non-overlapping domains. Patch pipette-filled astrocytes with two different coloured fluorescent dyes (OGB-1, a green fluorescent dye; Fura-red, a red fluorescent dye) illustrating the dense arrays of processes from each cell.

Astrocytic domains

Astrocyte domain organization in pathological states

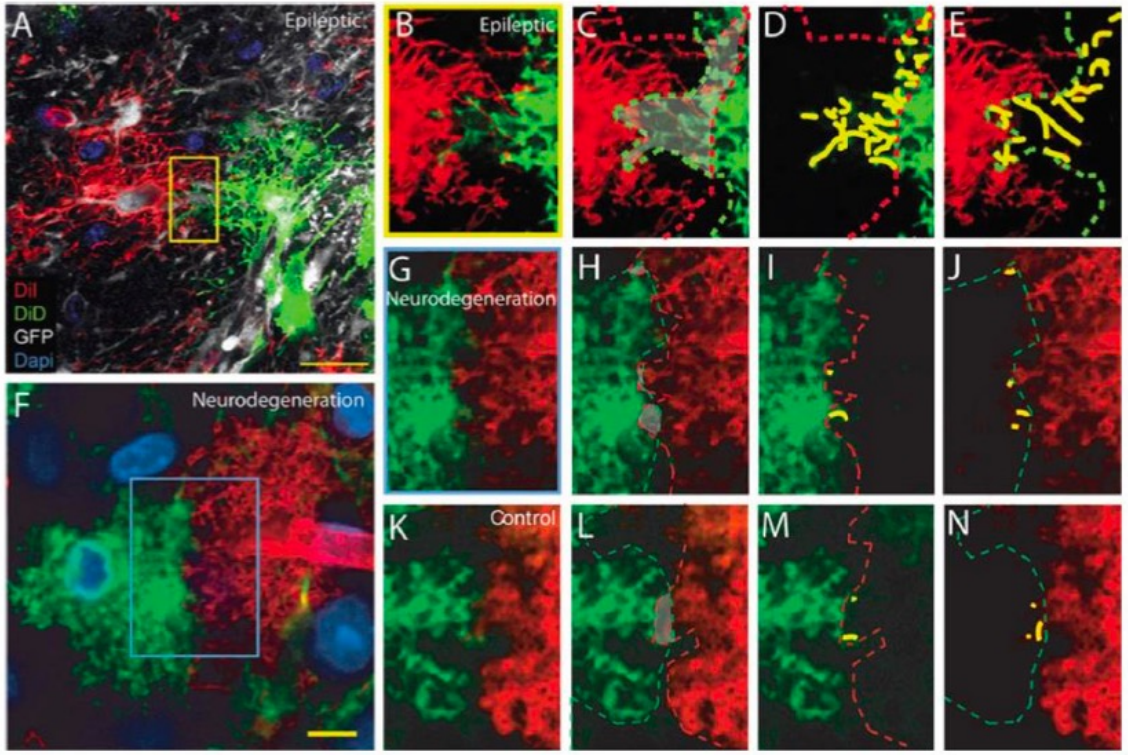
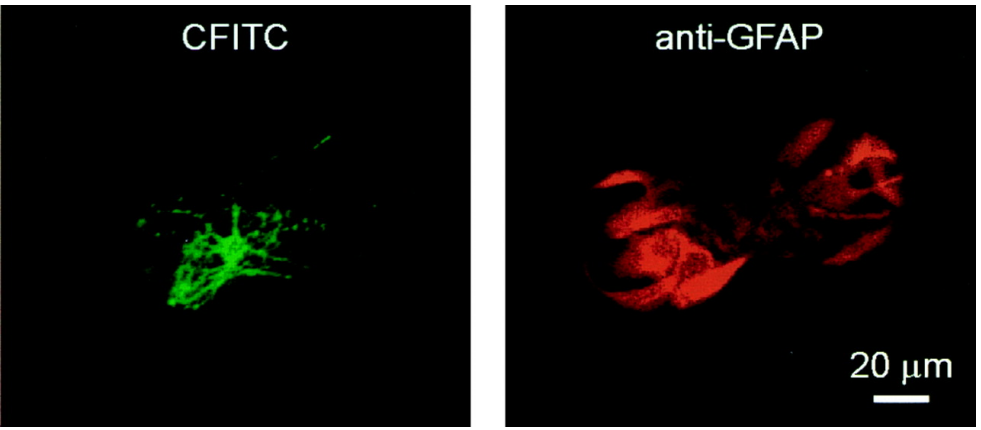


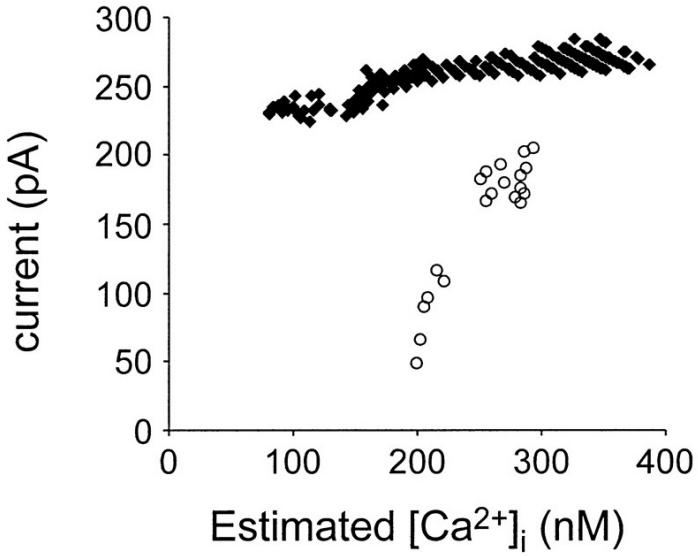
Fig. 2.
Astrocytic domain organization varies with pathology. The domain organization of protoplasmic astrocytes is lost in epileptic brains, but maintained in neurodegeneration. (**a**) Reactive astrocytes 1 week post-iron injection lose the domain organization. Diolistic labelling of the cortex of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

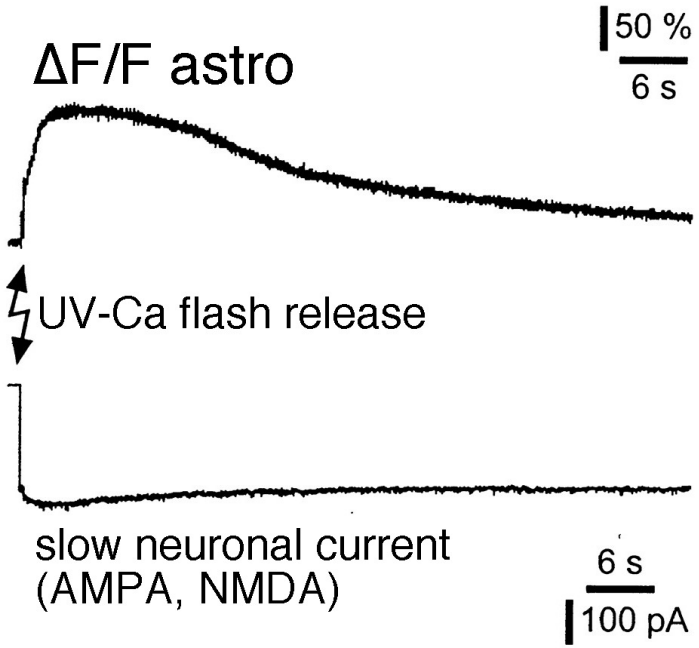
Oberheim et al., 2012
Methods Mol Biol. 814: 23–45.
doi:10.1007/978-1-61779-452-0_3

Astrocytes talk back to Neurons through the release of glutamate



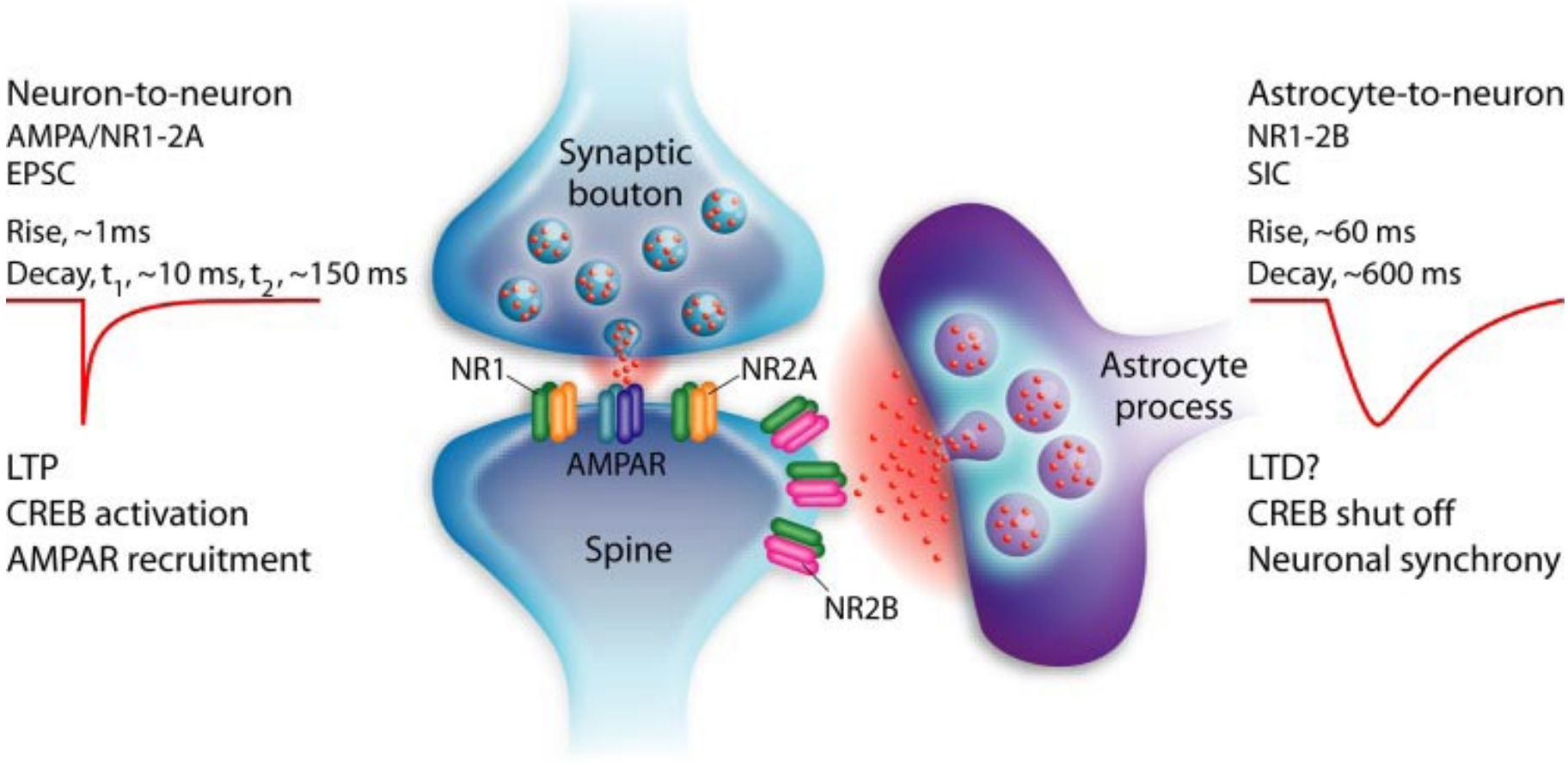
Micro-island: neuron on astrocytes (culture)
Parpura & Haydon, PNAS, 97 (2000)



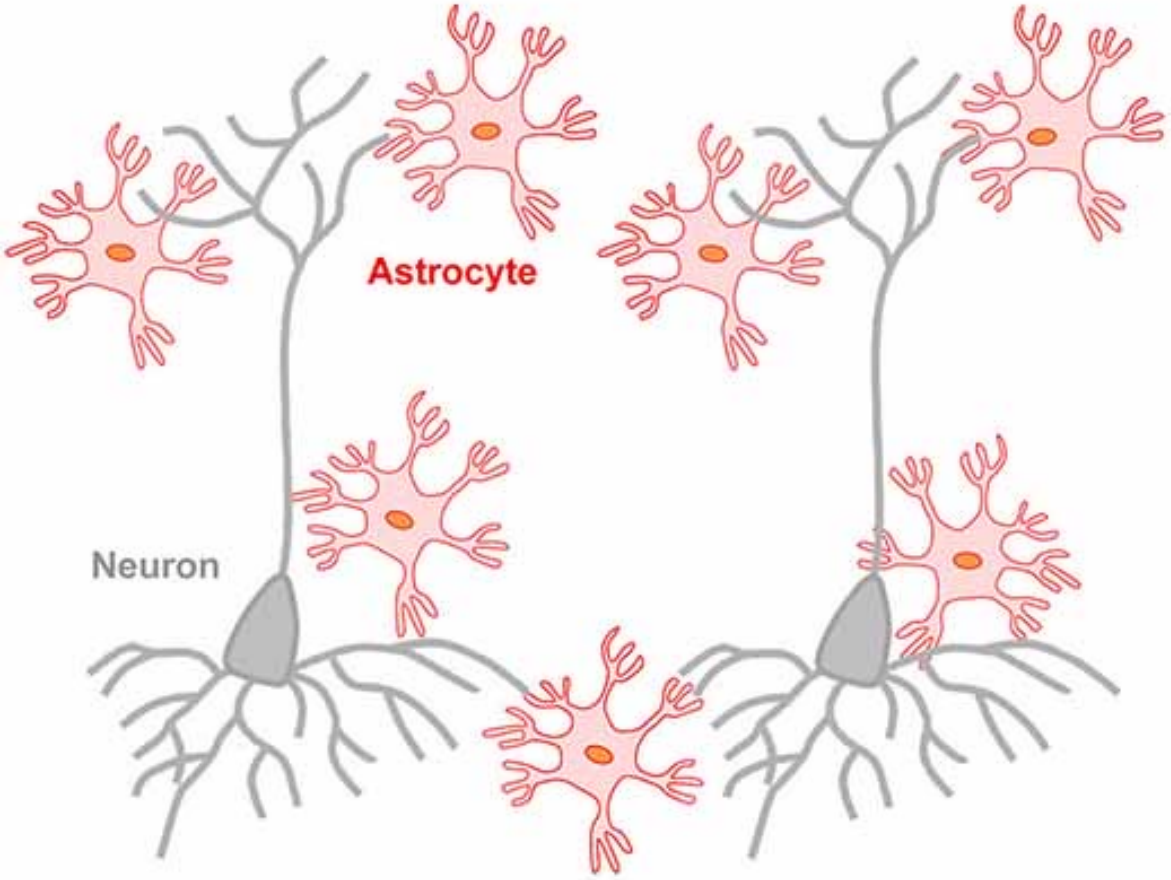


In slice: Fellin et al. , Neuron 43 (2004)

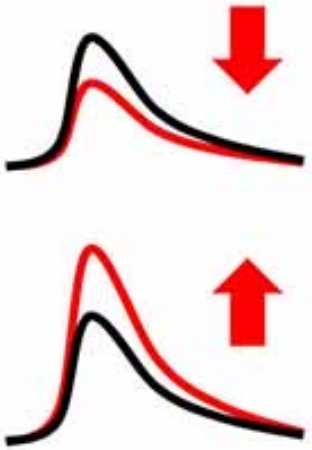
Glutamate released from presynaptic terminals and from astrocytes acts on distinct NMDA receptors.



NEURON *vs* NEURON-GLIA NETWORKS



Action potentials



Synaptic responses

GLIAL CONTROL OF NEURONAL EXCITABILITY AND SYNAPTIC TRANSMISSION

GLOBAL EFFECT ON NETWORK OPERATION

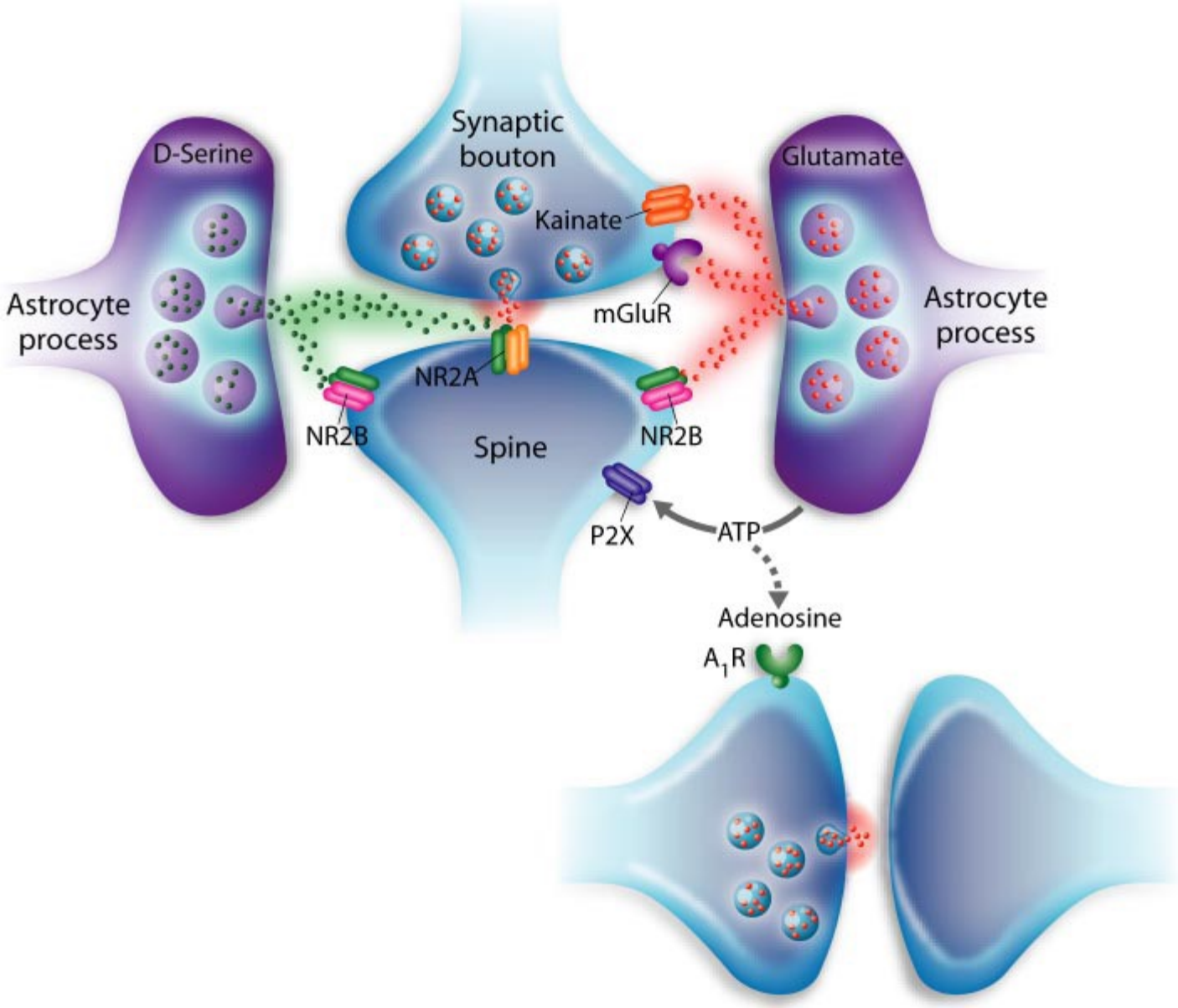
Reciprocal Communication

- Astrocytes detect released neurotransmitter, e.g. glutamate, by appropriate receptors
- This leads to an activation of the calcium signaling pathway
  - IP_3 levels go up
  - Calcium is released from internal stores
  - May propagate intra/intercellularly

Effects on the synapse

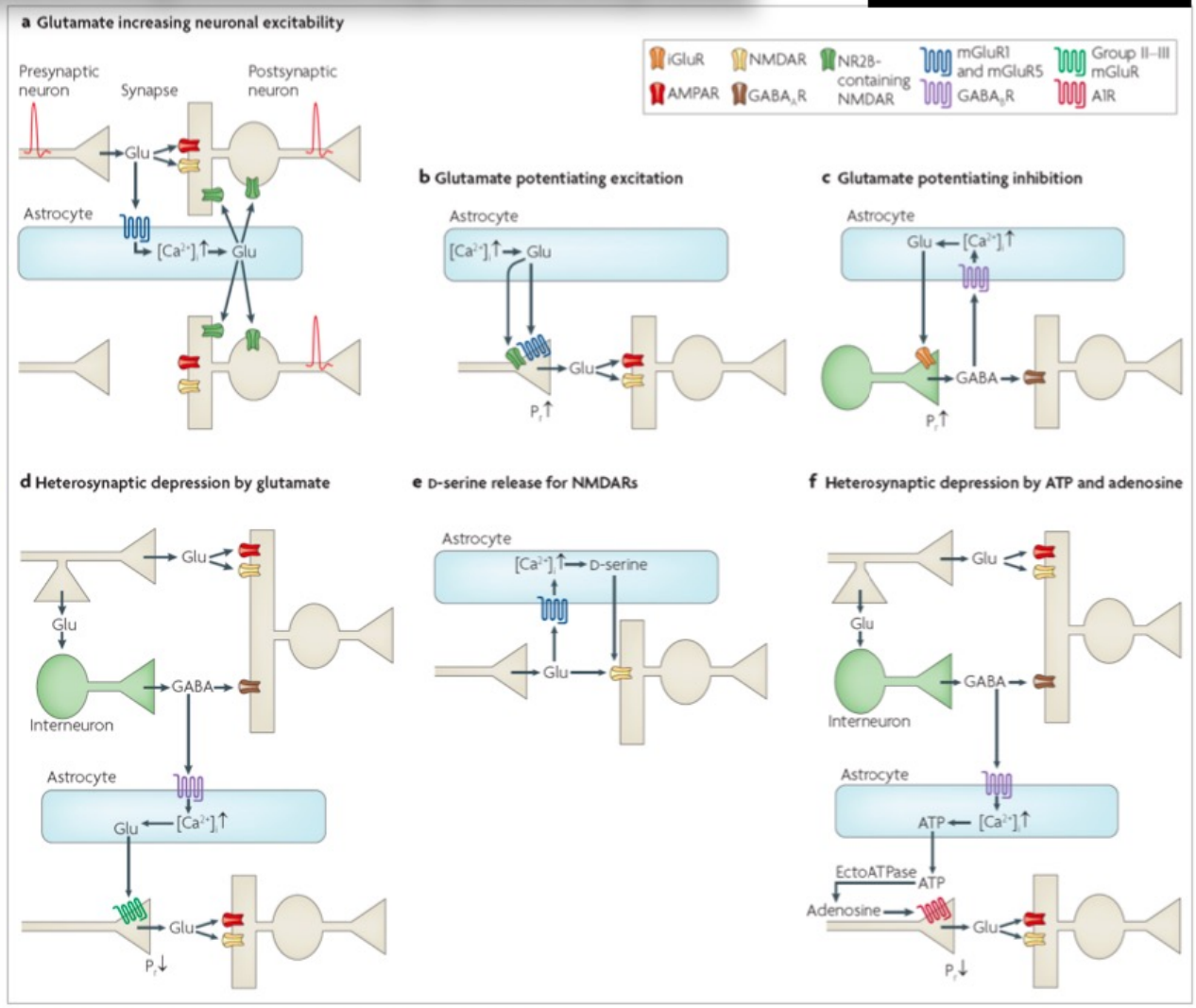
- Reduced amplitude of EPSC's
- Increased spontaneous releases
  - Depends on presynaptic mGluR and AMPA receptors
  - These are connected through the existence of a vesicle pool (that can become depleted)
- Increases fidelity of "weak" synapses
- There are also effects on the soma which will not be taken into account here, but are needed for a treatment of the full network.

Astrocyte-derived signals act both presynaptically and postsynaptically to regulate synaptic transmission



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).

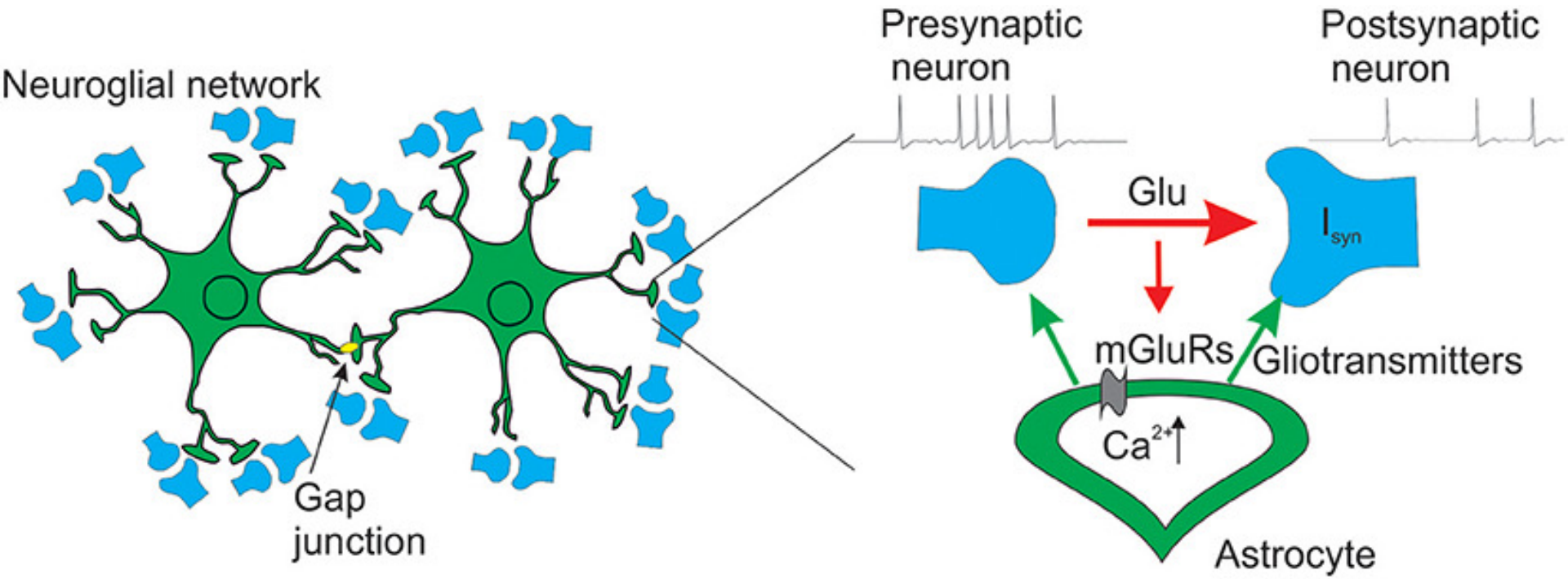
c | Glutamate release from astrocytes, triggered by GABA activating astrocyte GABAB receptors (GABABRs), **increases presynaptic GABA release.**

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.

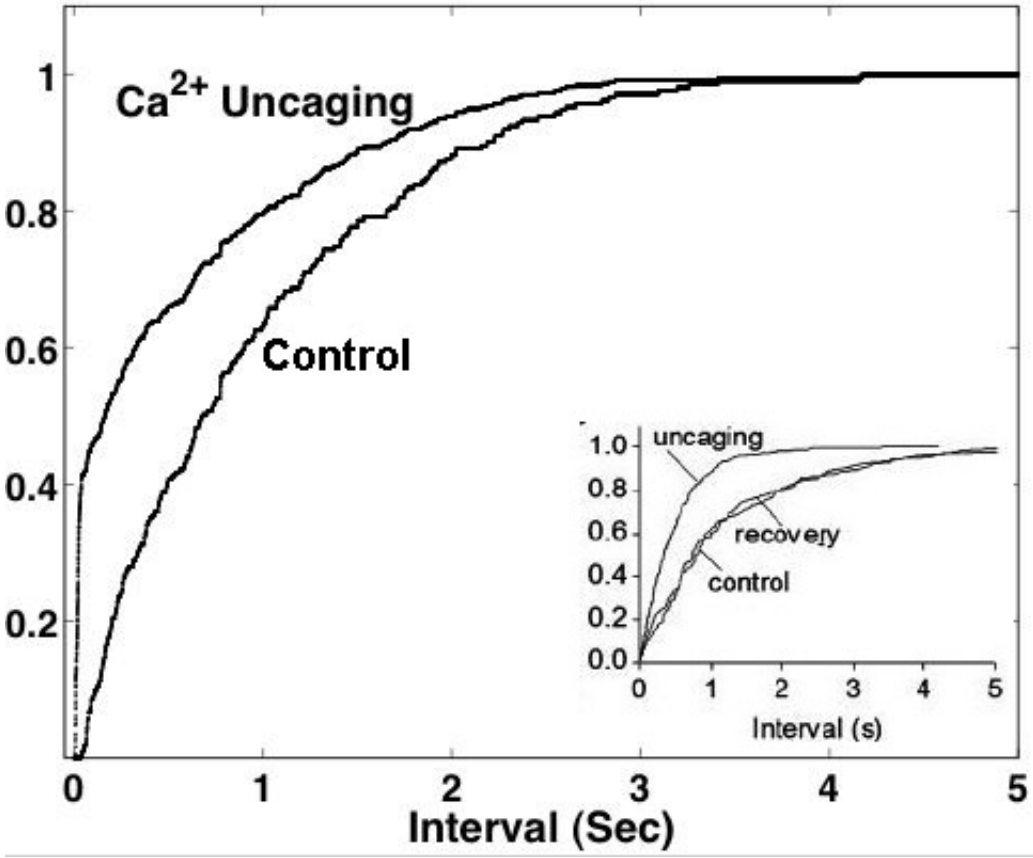
e | NMDAR activation regulated by Ca2+-dependent release of d-serine from astrocytes. d-serine activates neuronal NMDARs by binding to the NR1 subunit, thus controlling synaptic plasticity. In cultured cells, the [Ca2+]i increase that controls d-serine release has been shown to occur in response to the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

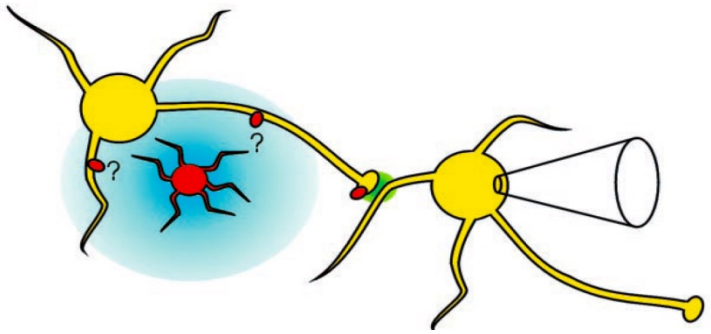
Astrocyte-derived signals act both presynaptically and postsynaptically to regulate synaptic transmission



Enhanced Spontaneous Activity due to Astrocytes

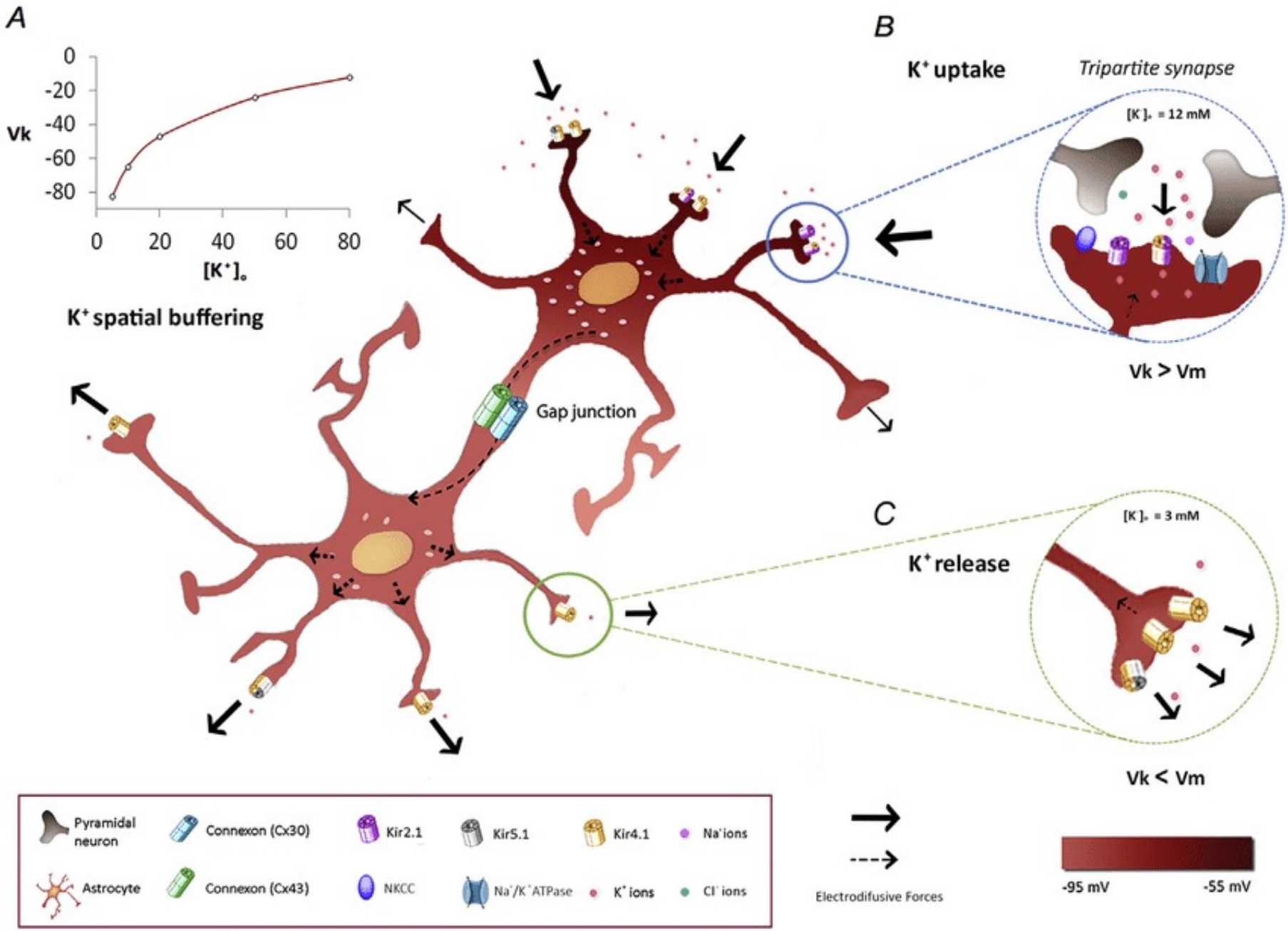


Liu et al. , PNAS, 2004

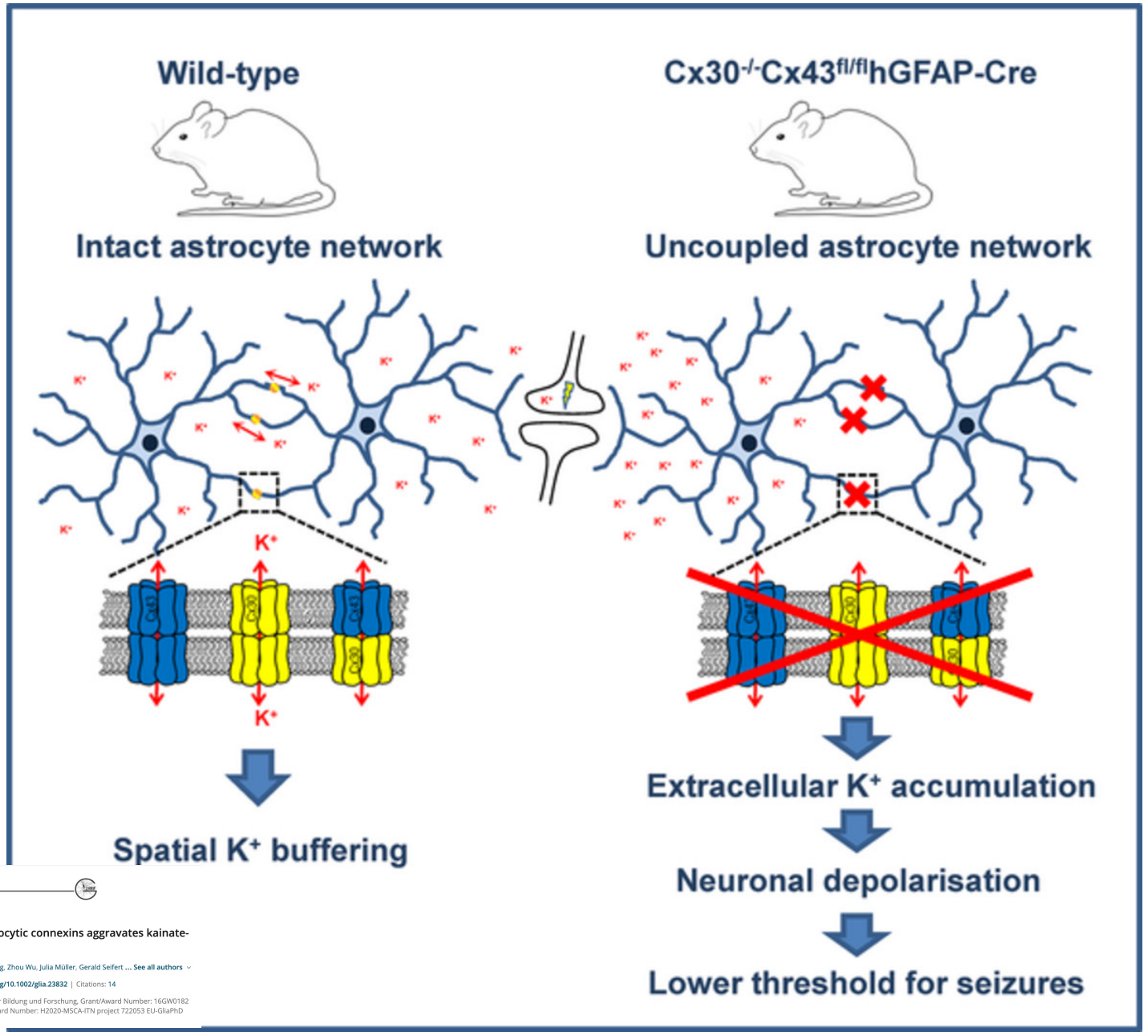


- Astrocyte is loaded with caged Ca^{2+}
- Upon photolysis, astrocyte released glutamate causes enhanced spontaneous activity.
- Leads to probability of current events (y axis) to shift to the left.

Extracellular ion buffering and neuronal activity



Extracellular ion buffering and epileptic activity



GLIA

RESEARCH ARTICLE | Open Access

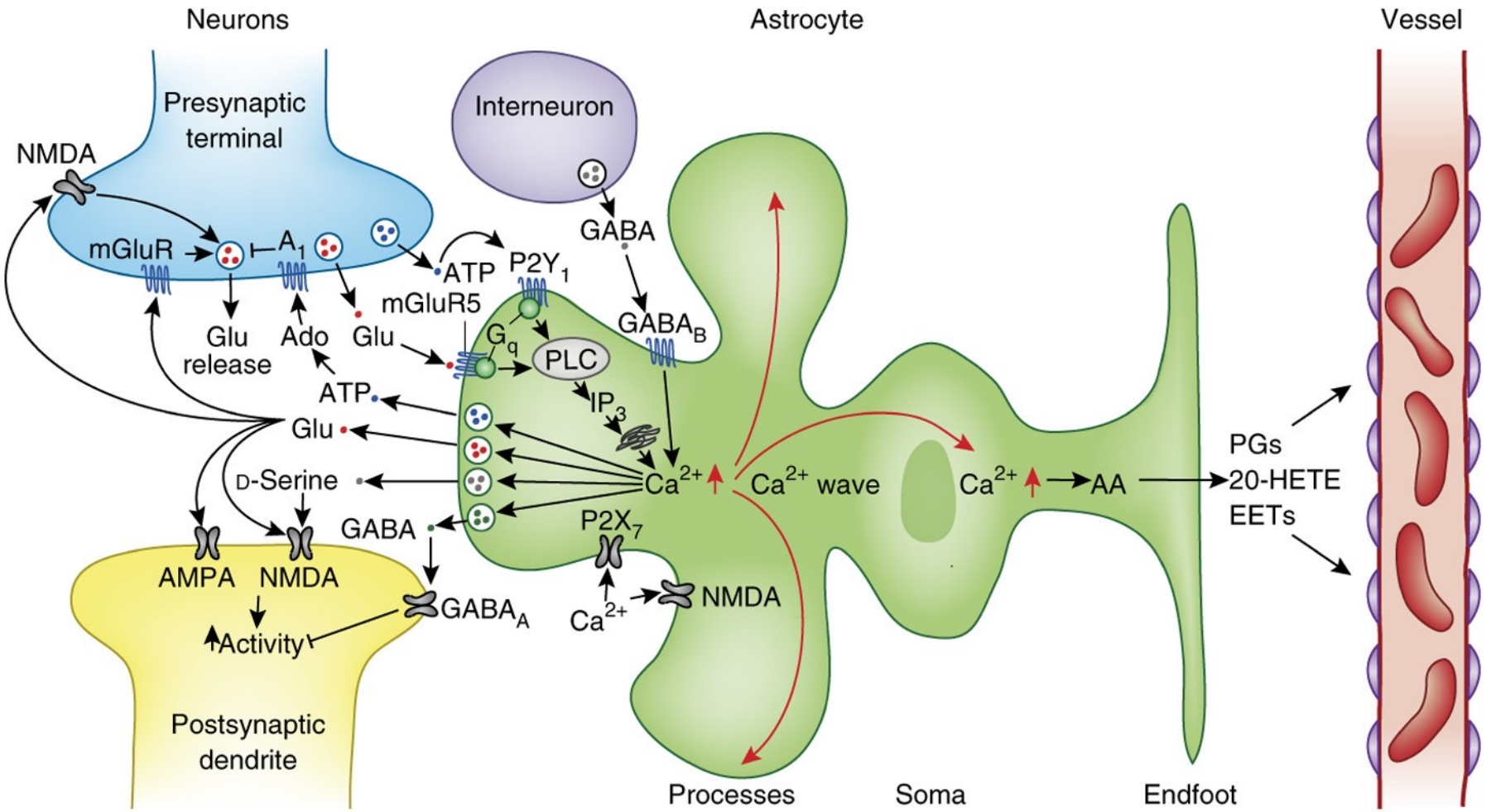
Constitutive deletion of astrocytic connexins aggravates kainate-induced epilepsy

Tushar Deshpande, Tingsong Li, Lukas Henning, Zhou Wu, Julia Müller, Gerald Seifert ... See all authors

First published: 02 April 2020 | https://doi.org/10.1002/glia.23832 | Citations: 14

Funding information: Bundesministerium für Bildung und Forschung, Grant/Award Number: 16GW0182 CONNEXIN; European Commission, Grant/Award Number: H2020-MSCA-ITN project 722053 EU-GliaPhD

Astrocyte calcium signaling: the third wave



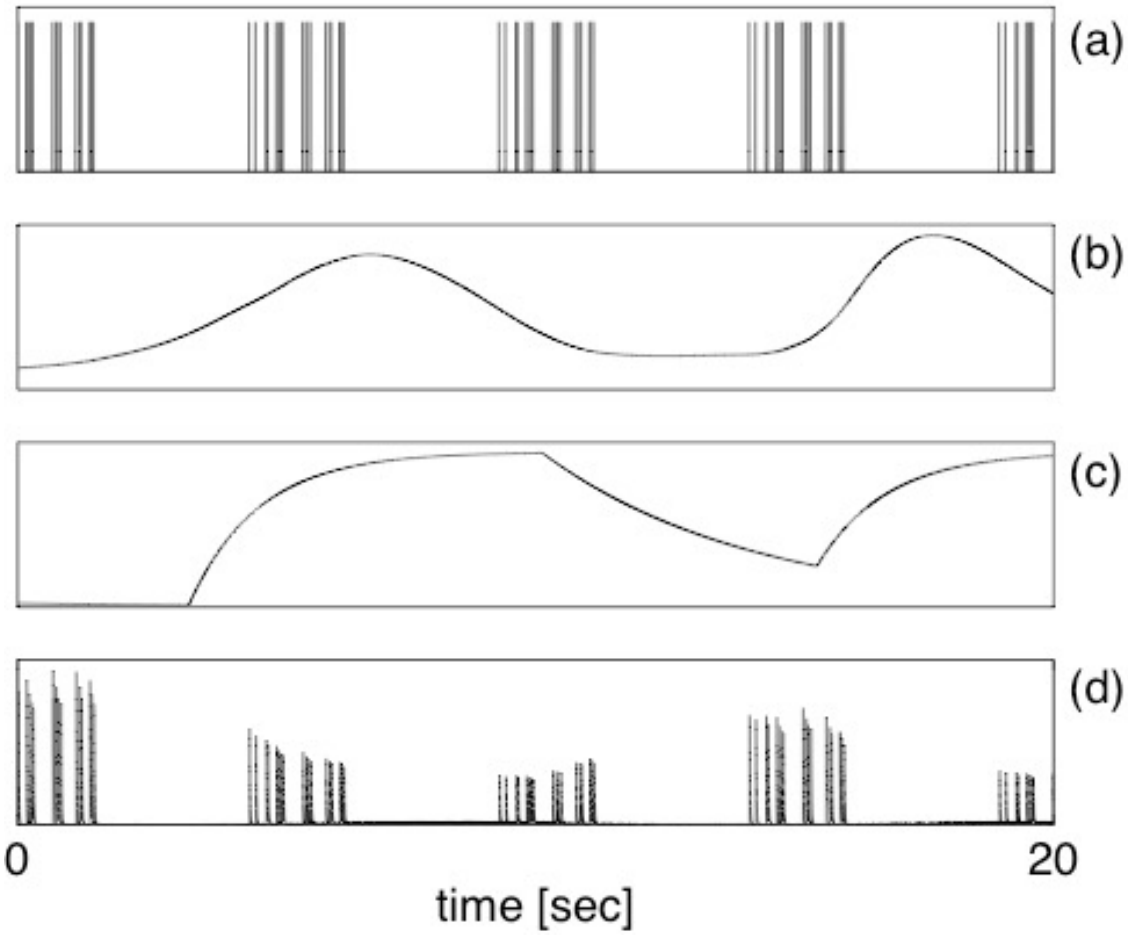
Modulated synapse

Input spike train

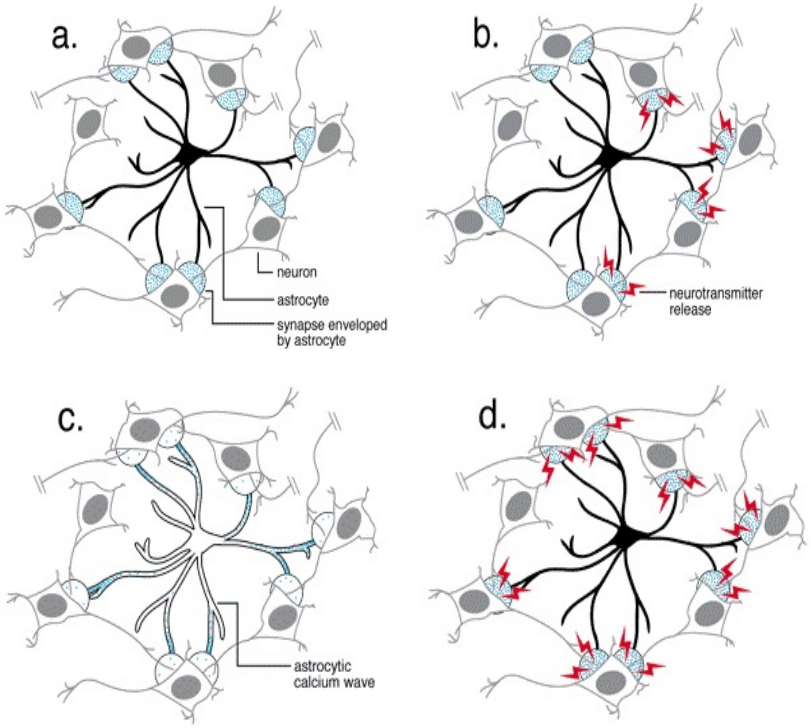
Glia calcium level

Synapse coupling factor

Postsynaptic current

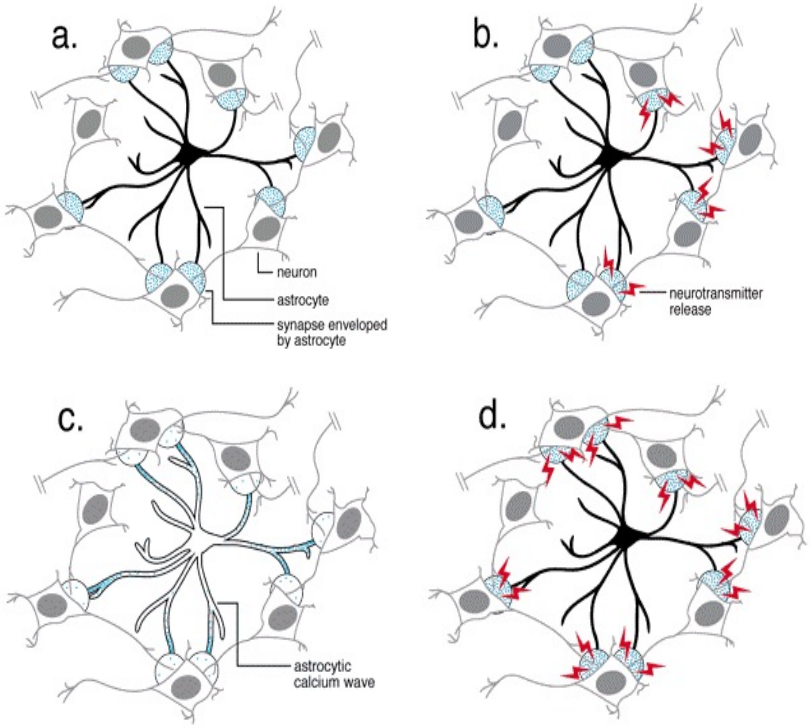


Synchronous Firing Groups- Astrocytic Regulation of Neural Networks



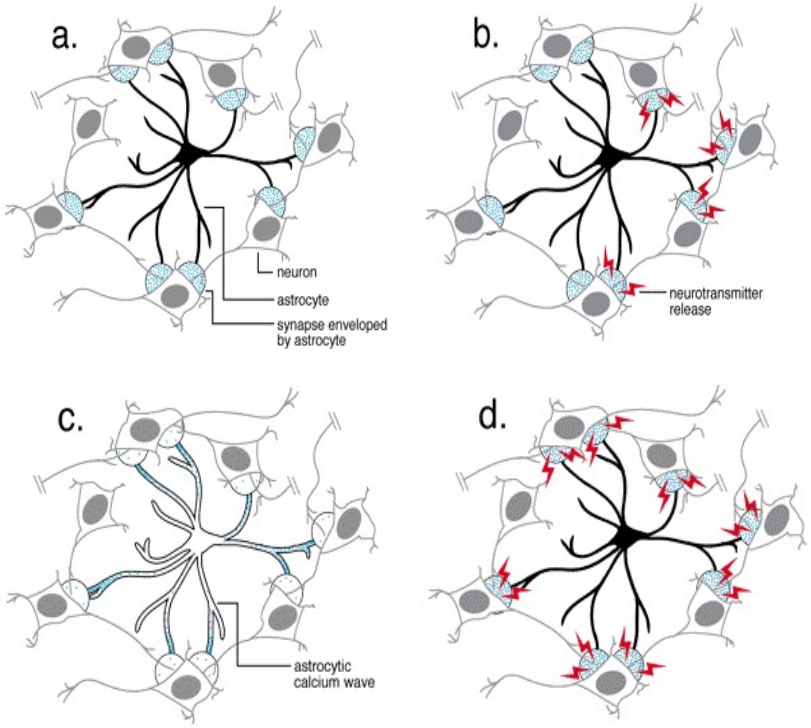
During a calcium wave, the synaptic environment changes dramatically. The astrocytic calcium wave reduces Ca2+ in the cleft. Decreased [Ca2+] in the cleft inhibits further neurotransmitter release, despite the arrival of action potentials. Only with termination of the astrocytic calcium wave will Ca2+ return to its original level in the synaptic cleft allowing neurotransmitter release at high levels.

Synchronous Firing Groups- Astrocytic Regulation of Neural Networks



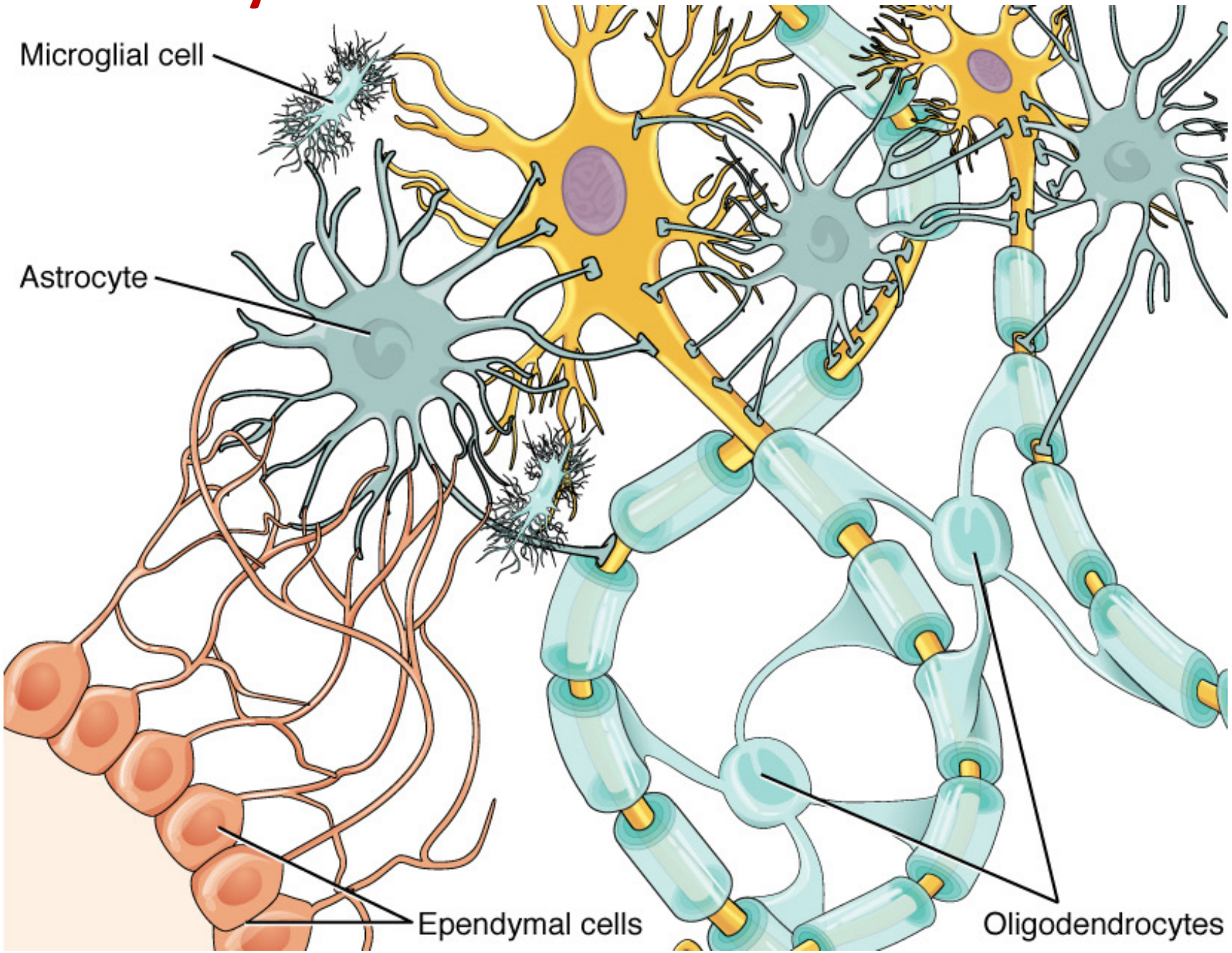
By simultaneously regulating neurotransmission in all of the synapses an astrocyte has enveloped, the astrocytic calcium wave may coordinate synapses into synchronously firing groups. Thus, all of the synapses enveloped or partially enveloped by an astrocyte may be within that astrocyte's domain of synaptic influence. In effect, one group of neurons could possibly influence another distant group of neurons through strictly astrocytic pathways.

Synchronous Firing Groups- Astrocytic Regulation of Neural Networks



The implications of this are enormous; entire models of cognitive functioning could possibly be infuenced by these astrocyte to neuron communications.

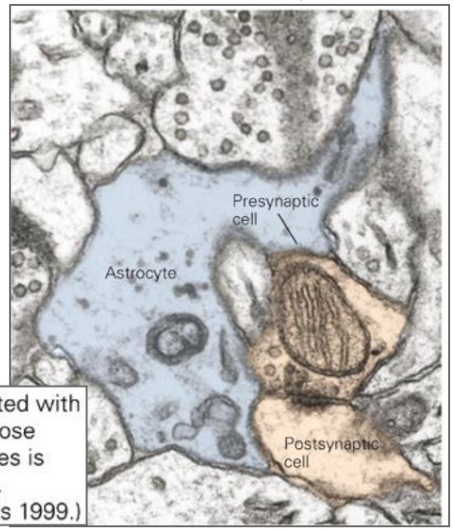
Pan astrocytic network….



Pan astrocytic network….

- 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.)

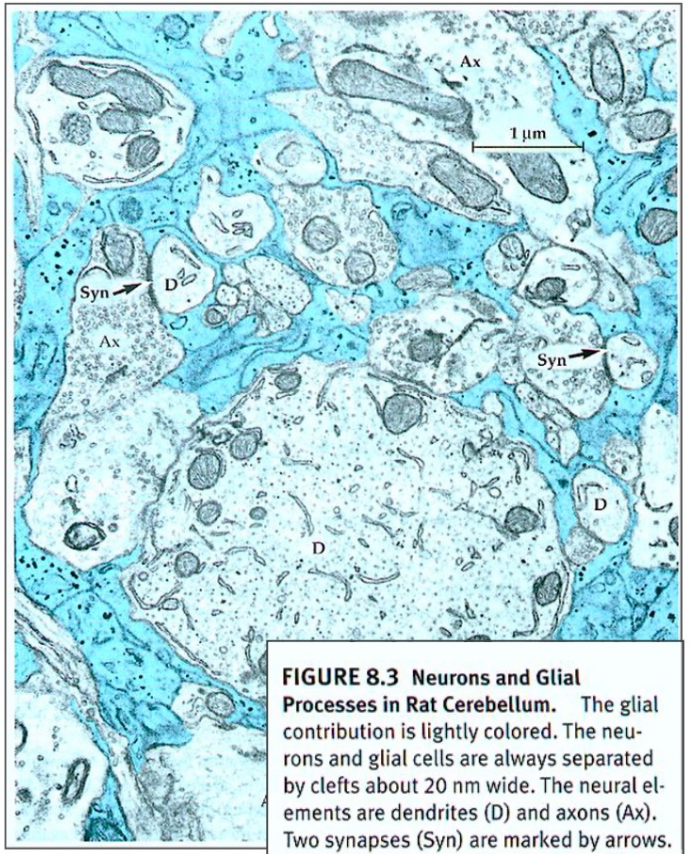
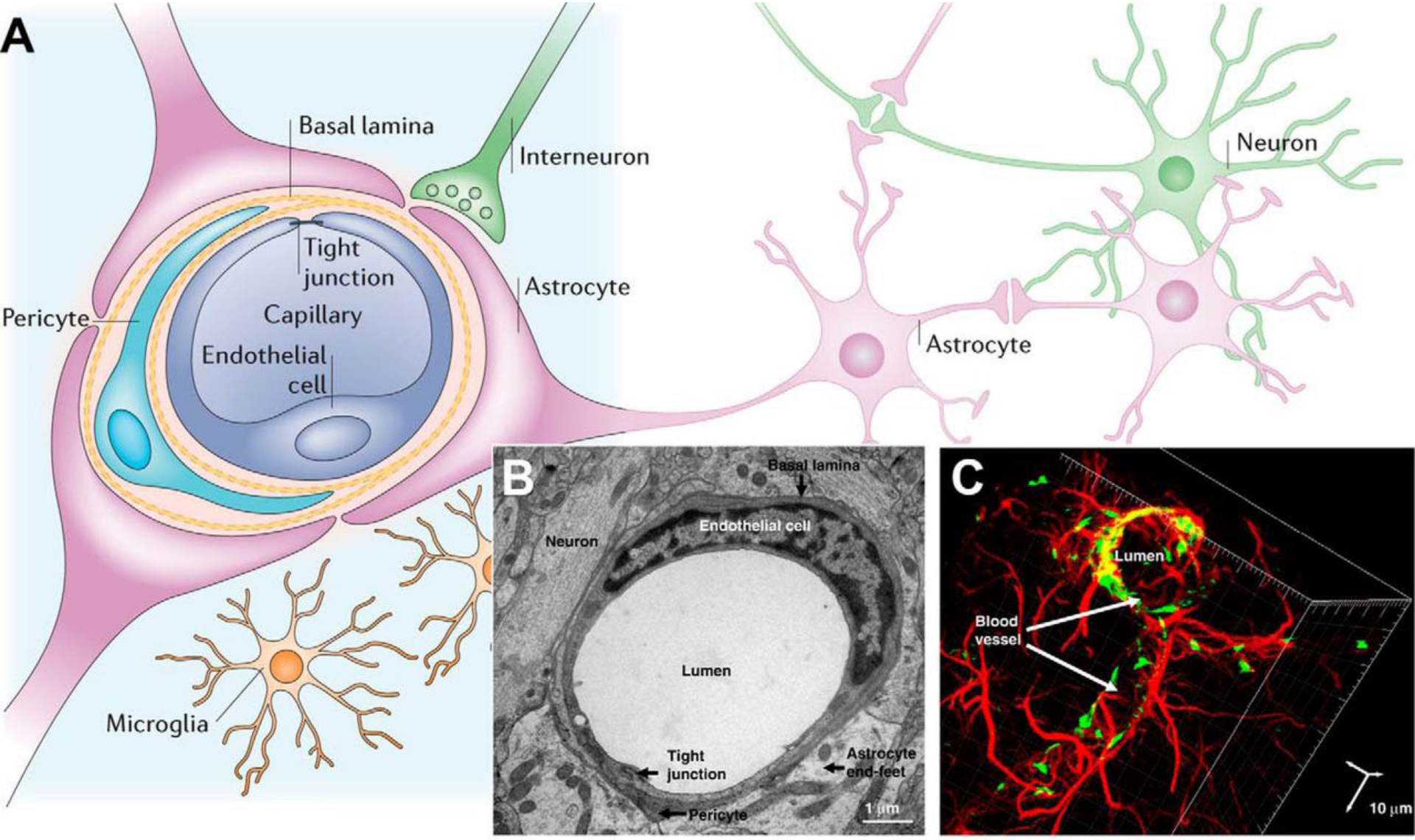


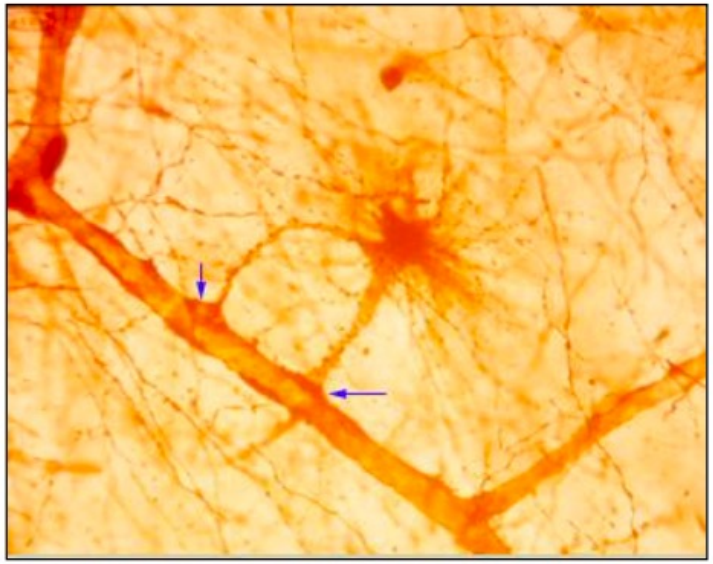
FIGURE 8.3 Neurons and Glial Processes in Rat Cerebellum. The glial contribution is lightly colored. The neurons and glial cells are always separated by clefts about 20 nm wide. The neural elements are dendrites (D) and axons (Ax). Two synapses (Syn) are marked by arrows. (After Peters, Palay, and Webster,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

Astrocytes and brain homeostasis: regulation of blood flow

Astrocytes and brain homeostasis: regulation of blood flow

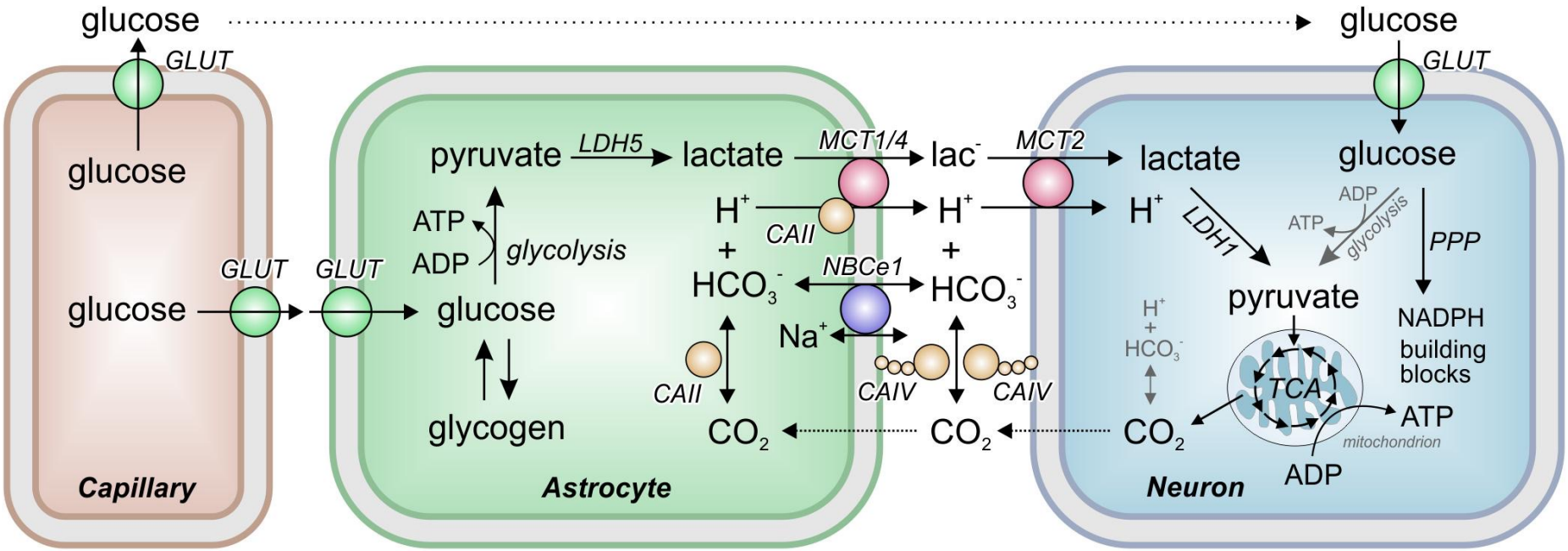


Astrocytes and brain homeostasis: regulation of blood flow



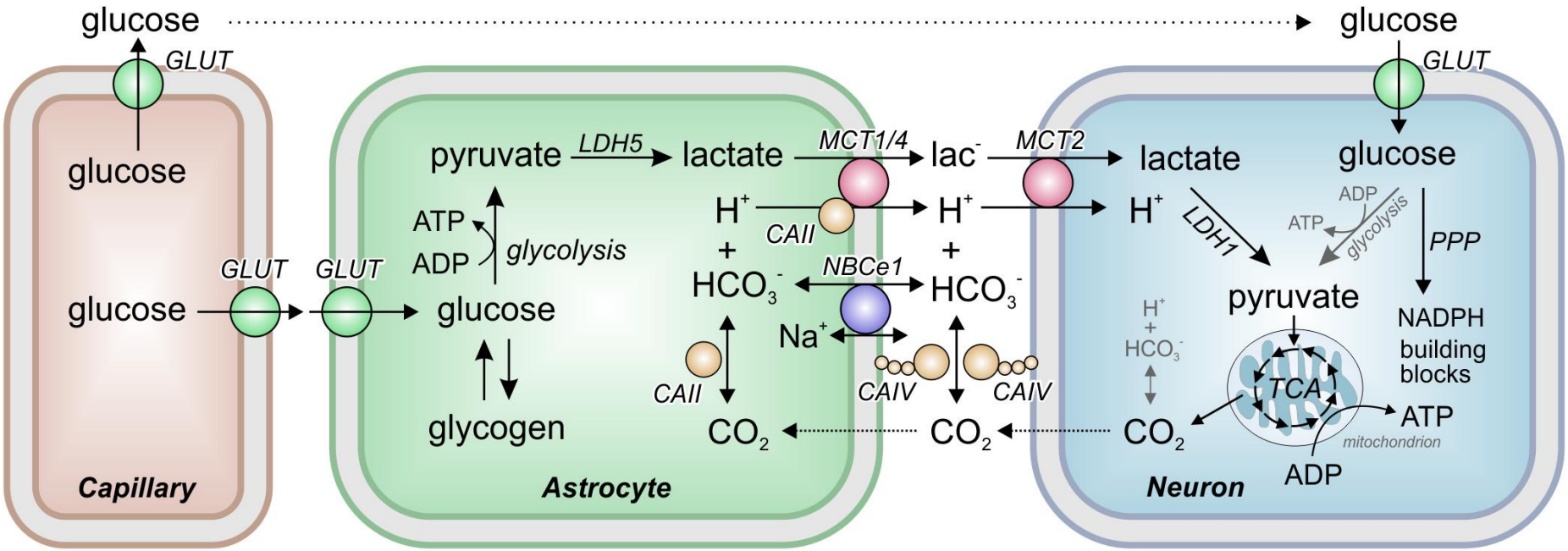
- Numerous fine processes of astrocytes form close associations with capillaries and neurons.
- Enhanced neuronal activity causes astrocytes to signal to blood vessels for regional increases in blood flow.
- Results in enhanced delivery of oxygen and glucose to the active brain regions.

Astrocyte-Neuron Lactate Shuttle



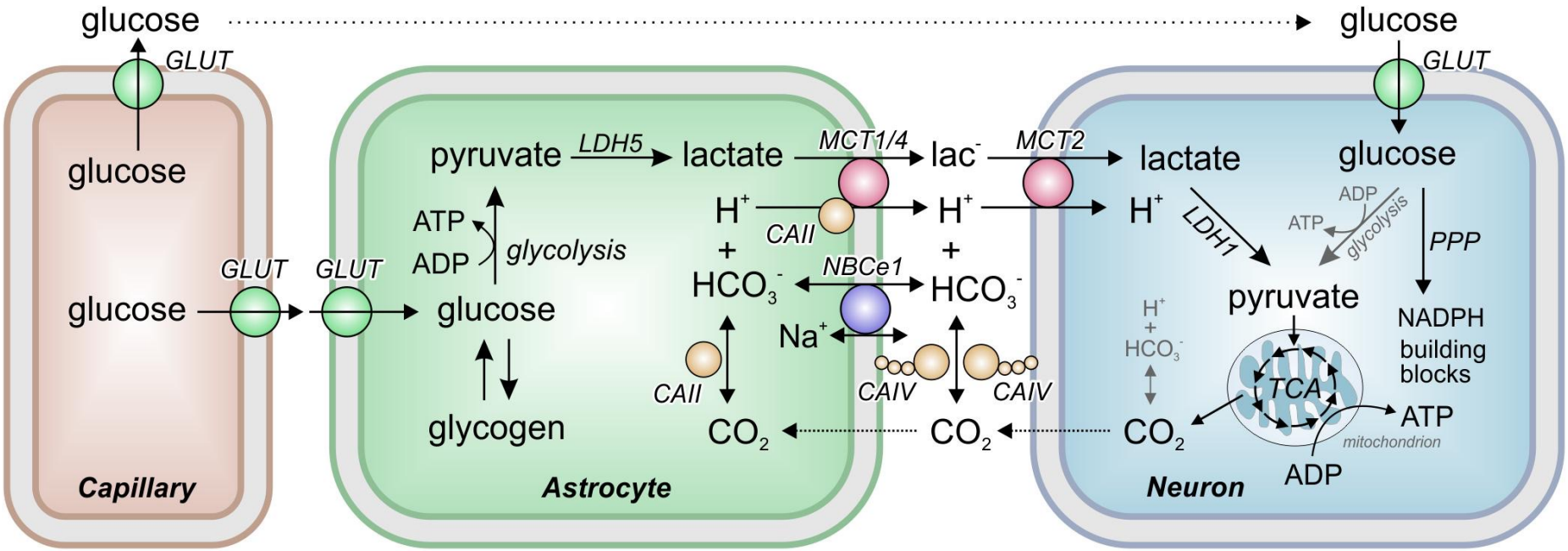
- Astrocytes take up glucose from the blood capillaries via glucose transporters (GLUTs).
- In astrocytes, glucose is either stored as glycogen or metabolized to pyruvate in the glycolysis.
- Pyruvate is then converted to lactate by the oxidoreductase lactate dehydrogenase (LDH) isoform 5 (LDH5).

Astrocyte-Neuron Lactate Shuttle



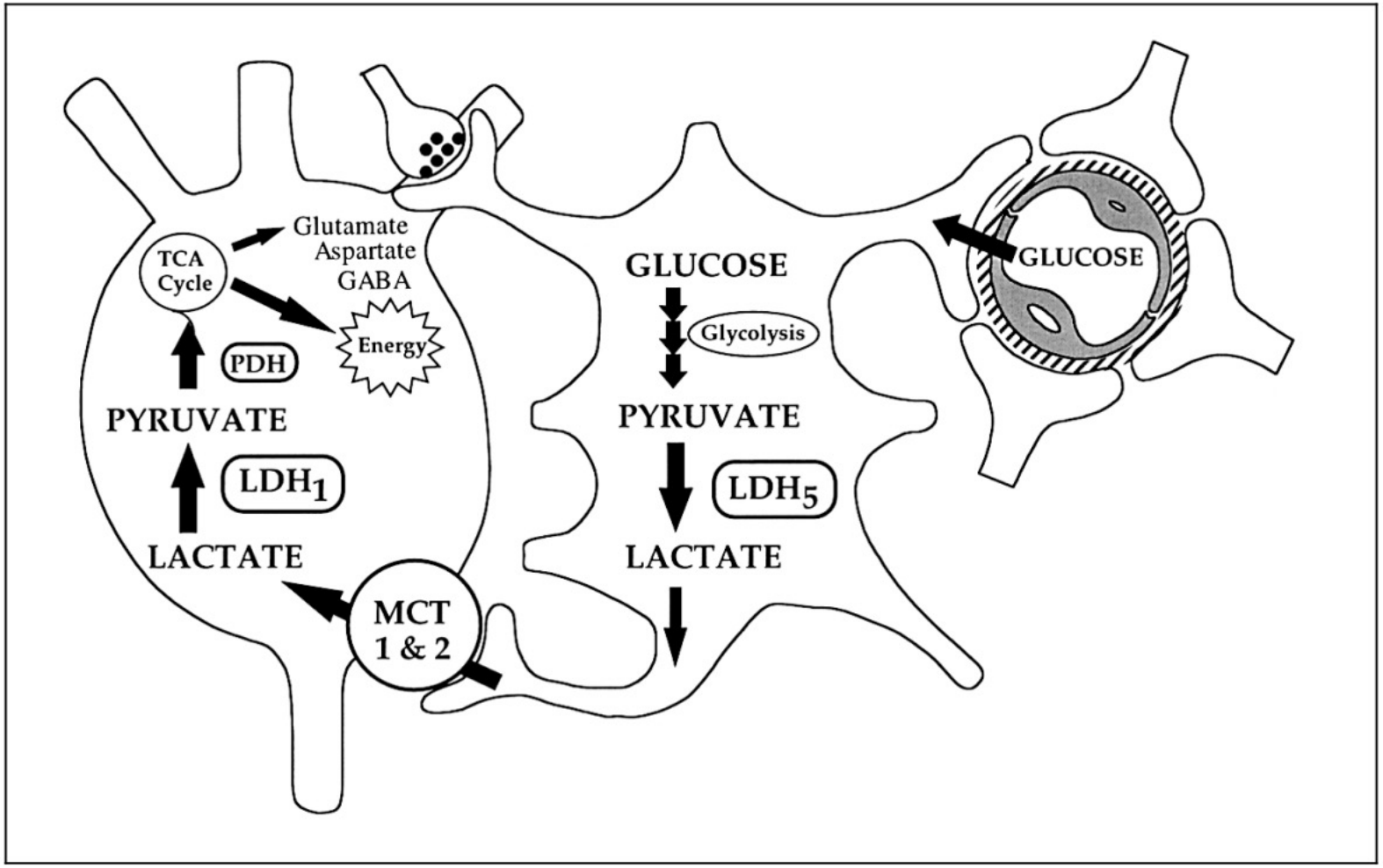
- The lactate is transferred from astrocytes to neurons by the monocarboxylate transporters (MCTs) MCT1, MCT2, and MCT4 in cotransport with a proton.
- MCT transport activity was found to be facilitated by interaction with the carbonic anhydrases (CAs) CAII and CAIV, which catalyze the equilibrium of H^+, HCO_3^- and CO_2 both intra- and extracellularly, and by the activity of the electrogenic sodium-bicarbonate cotransporter NBCe1.

Astrocyte-Neuron Lactate Shuttle

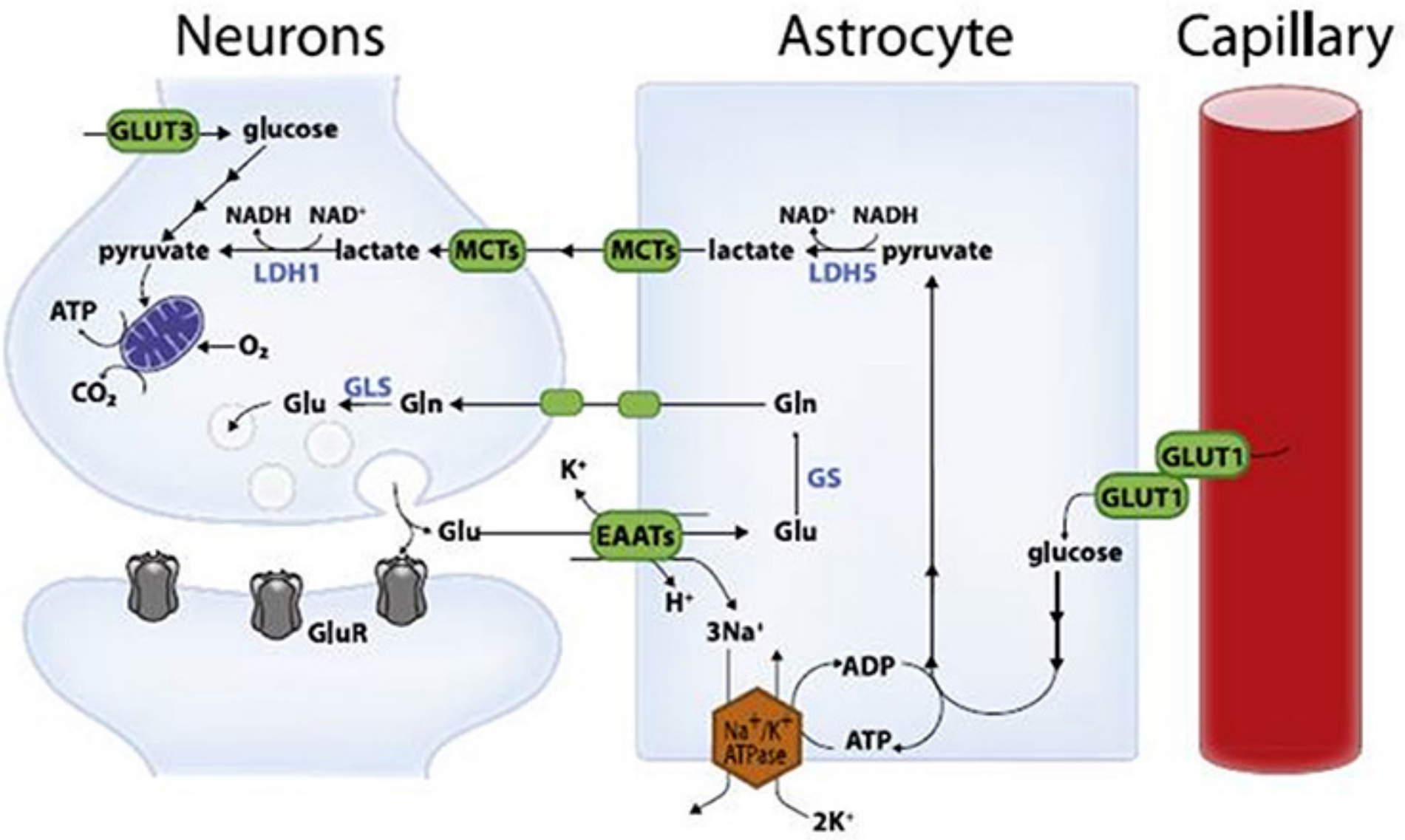


- In neurons, lactate is converted back to pyruvate by LDH1 and transferred into mitochondria for aerobic energy production in the tricarboxylic acid cycle (TCA).
- In addition, glucose is directly taken up into neurons where it can either serve as energy source in the glycolysis or is shuttled into the pentose phosphate pathway (PPP) for production of NADPH and cellular building blocks like ribose-6-phosphate.

Activity-Dependent Astrocyte-Neuron Lactate Shuttle



Activity-Dependent Astrocyte-Neuron Lactate Shuttle



Astrocytes and brain homeostasis: regulation of blood flow

As **Neural activity** increases there is an increased **Energy requirement**

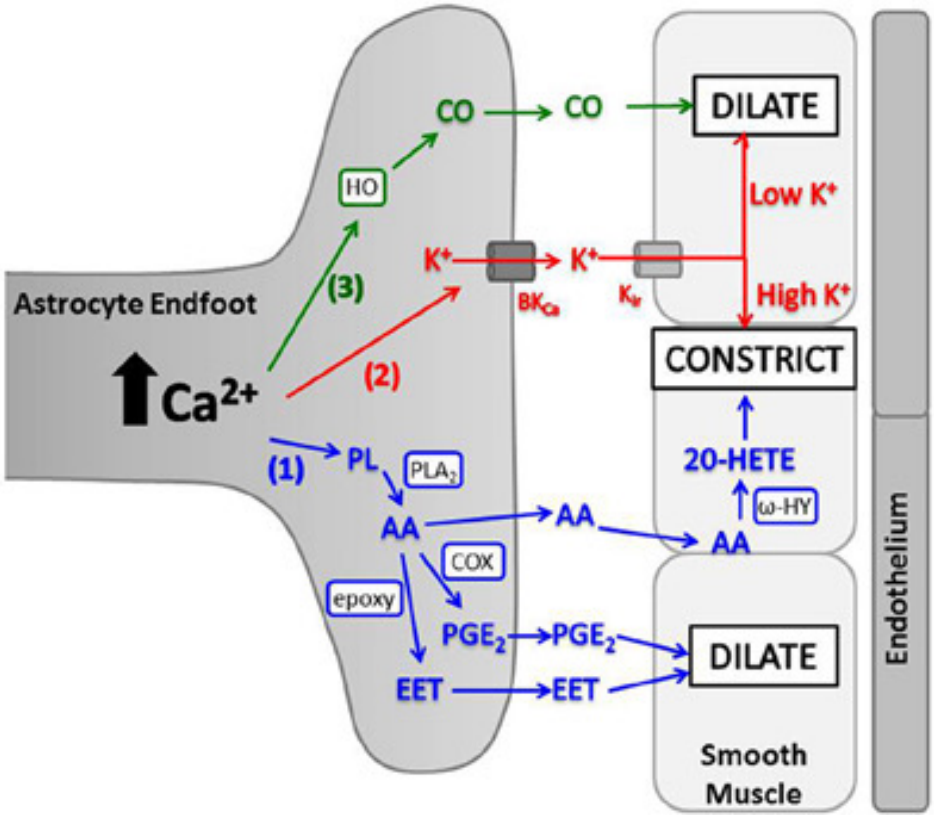
To solve this…

Astrocytic uptake of Glutamate leads to> ADP leads to> Glycolysis within Astrocytic endfeet **which finally leads to> Lactate delivered to neuron**

=Energy demand met! But what about OXYGEN? Waste? Other nutrients?

With increased neural activity, there MUST be an increase in **LOCAL CIRCULATION OF BLOODFLOW**

Astrocytes and brain homeostasis: regulation of blood flow



Astrocyte intracellular Ca^{2+} elevations trigger release of vasoactive molecules. **(1)** PLA_2 is activated by Ca^{2+} and converts phospholipids (PL) to AA. AA is metabolized in astrocyte endfeet to PGE_2 (by COX) or EET [by cytochrome P450 epoxygenase (epoxy)] which dilate arterioles, or AA can diffuse to smooth muscle where ω-hydroxylase (ω-HY) converts it to 20-HETE and causes constriction. **(2)** K^+ is released from astrocyte endfeet through BK_{Ca}, and the amount of K^+ released is directly proportional to astrocyte Ca^{2+} level. K^+ is taken up into smooth muscle through K_{ir} and causes dilation at low concentrations and constriction at high concentrations. **(3)** HO is activated by Ca^{2+} and produces CO, which diffuses to smooth muscle and triggers dilation.

Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation

↑ Neural Activity

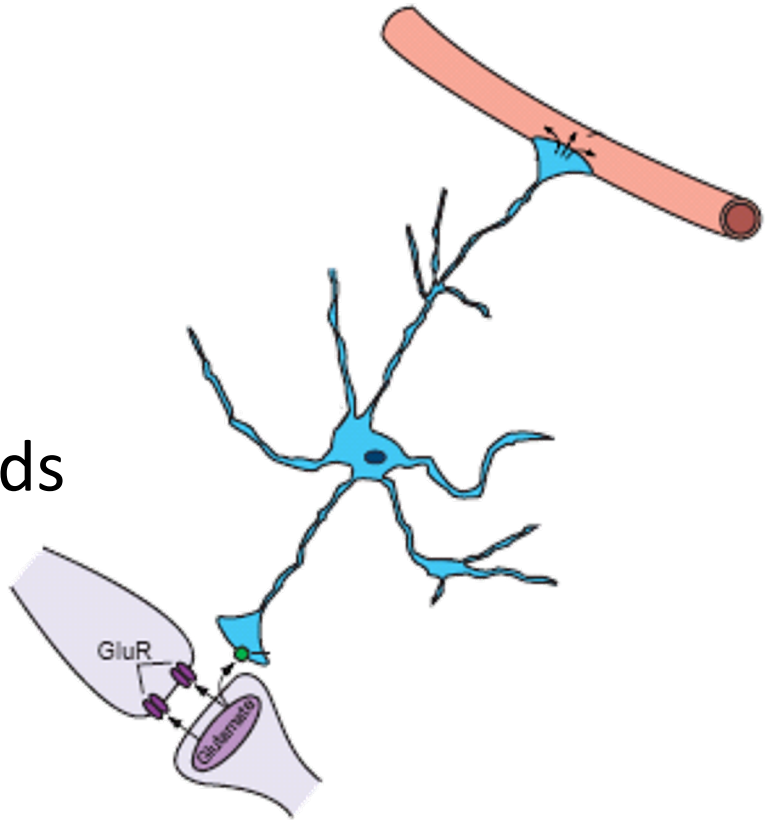
↑ Ca++ propagation throughout astrocytic syncytium

↑ [Ca++] at endfeet attached to endothelial cells

↑ Vesicular release of prostanoids

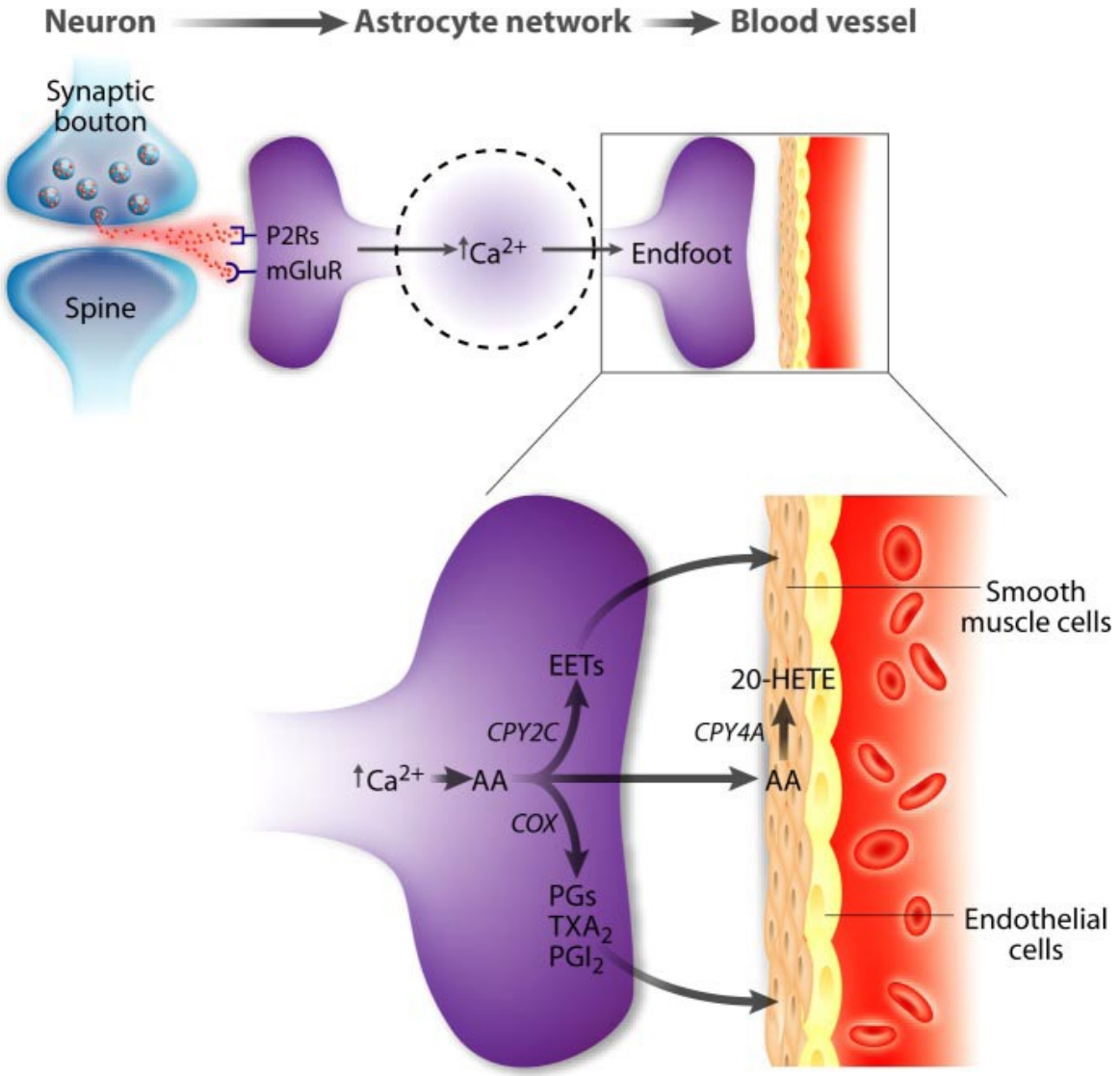
↑ Relaxation of capillary walls; decrease in vascular tone

↑ **Bloodflow**

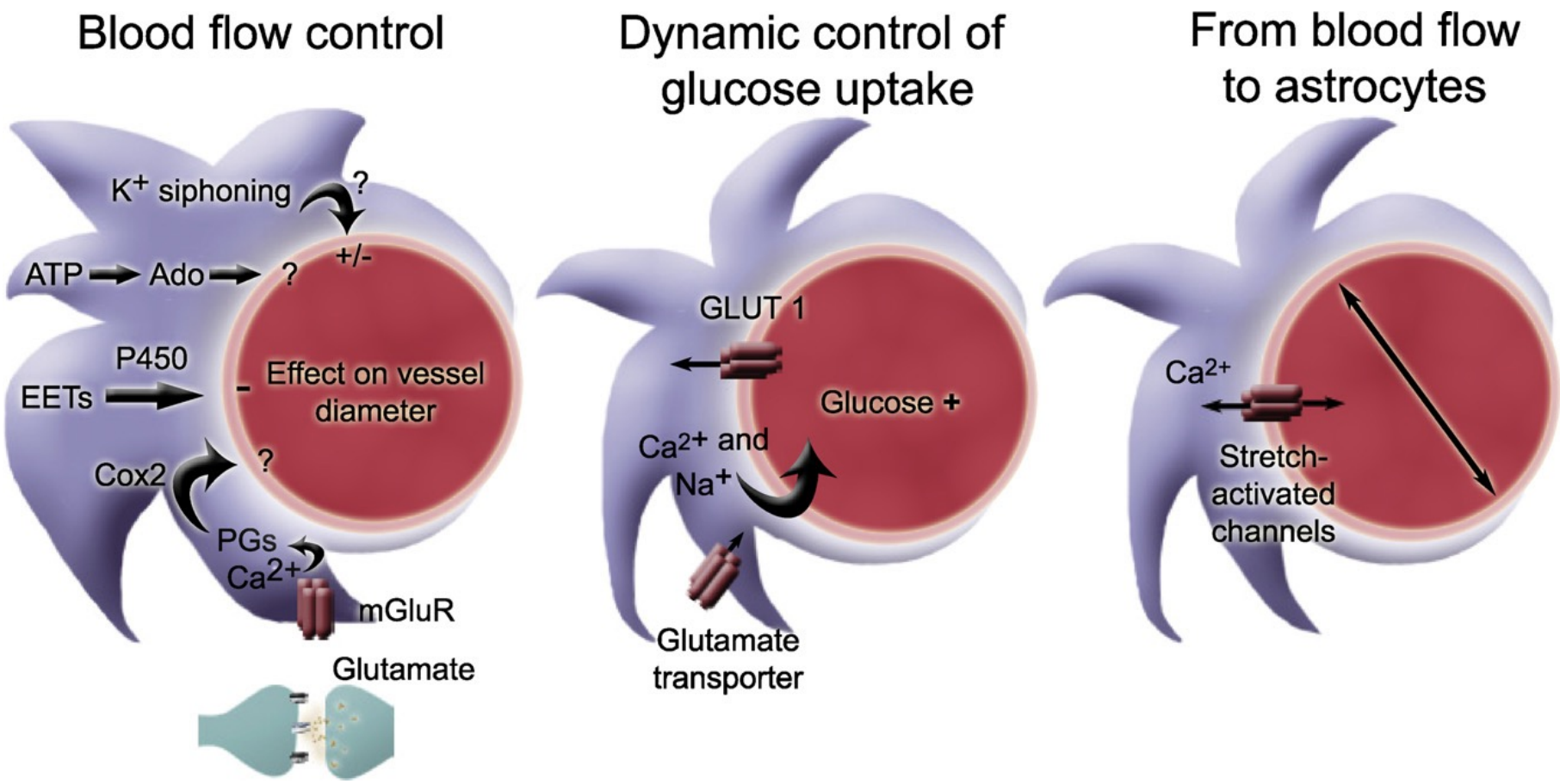


Zonta et al., 2003

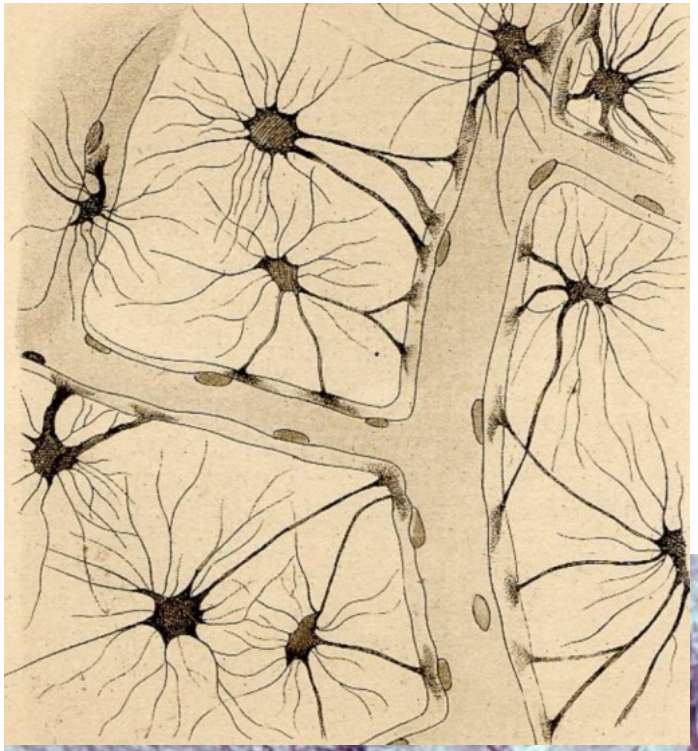
Neuronal synaptic activity can act through the astrocyte network to regulate the cerebrovasculature.

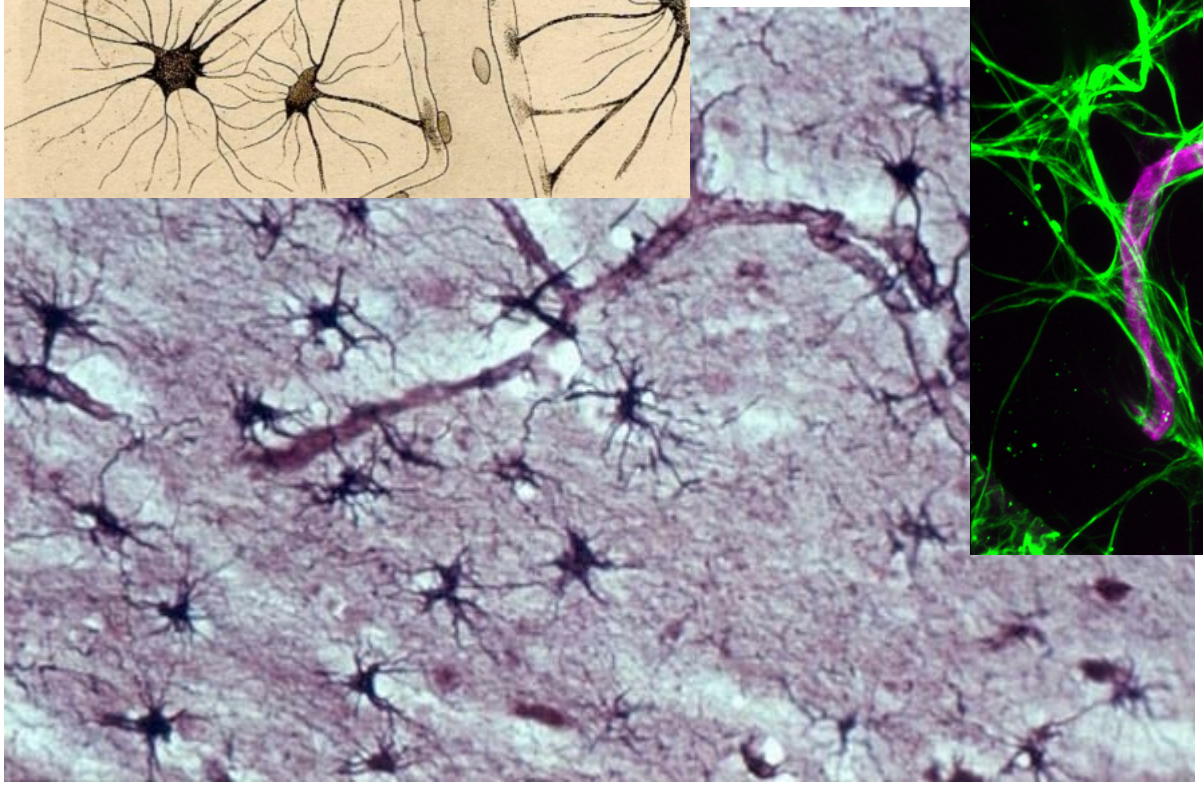


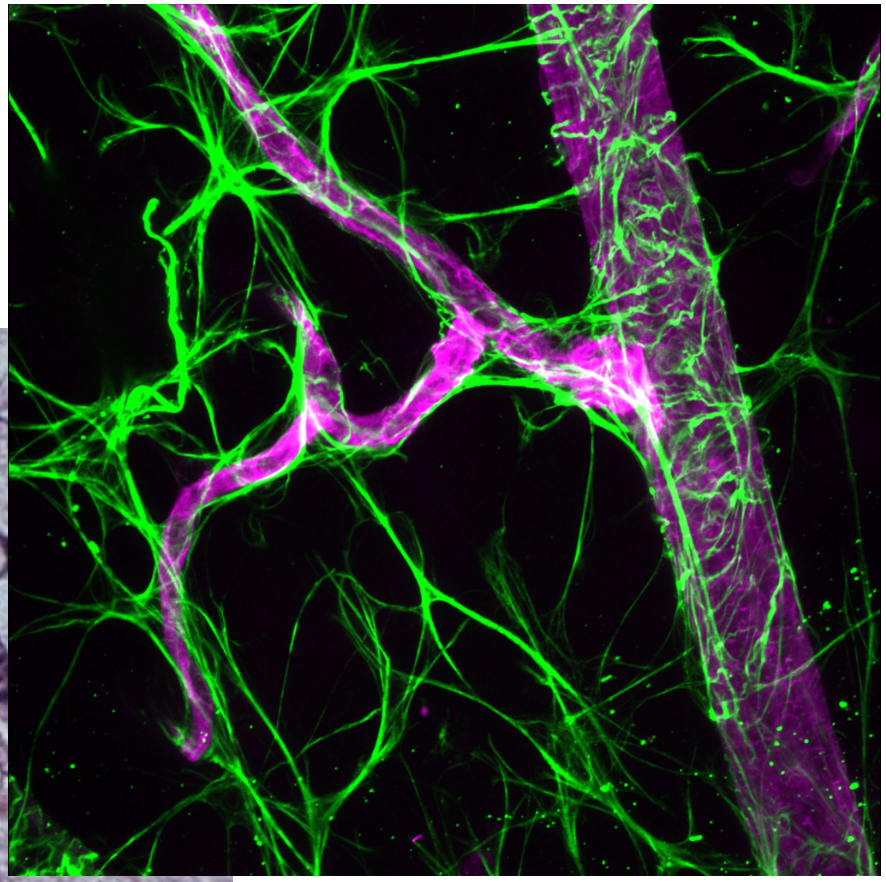
Control of vascular tone



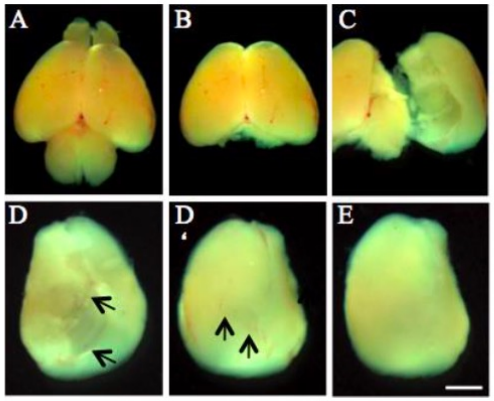
Astrocyte/capillary interactions: foot process





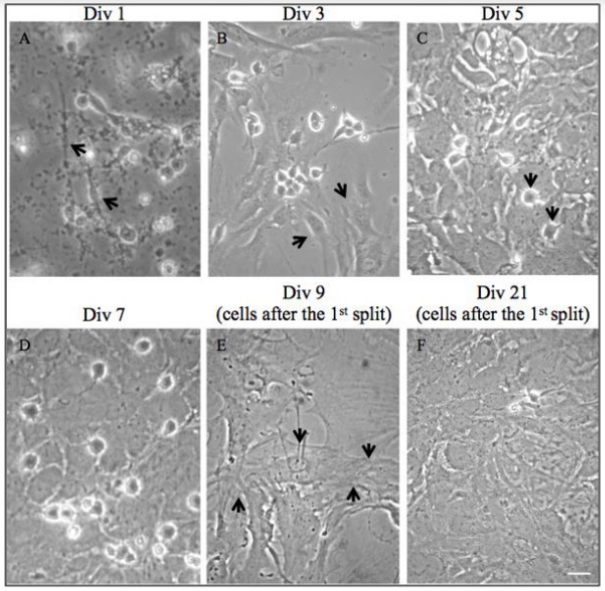


Isolation and Culture of Mouse Cortical Astrocytes

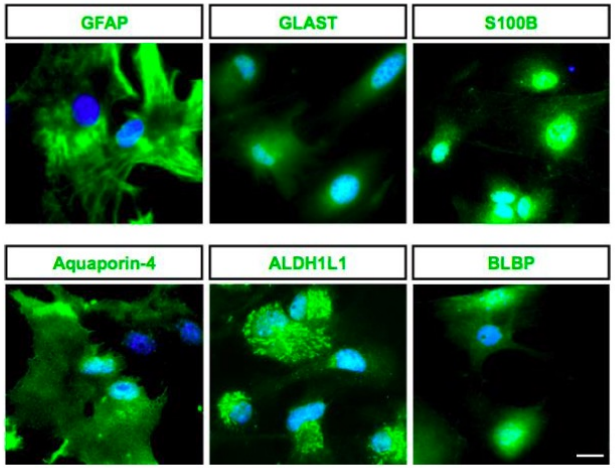


Dissection of postnatal (P3) mouse cortex. A) Whole brain. B) Brain after removal of olfactory bulbs and cerebellum. C) Isolation of cortices by peeling off the plate-like structure of the cortex from the brain. D, D') Cortex from ventral and dorsal site with meninges (black arrows indicate meningeal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

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

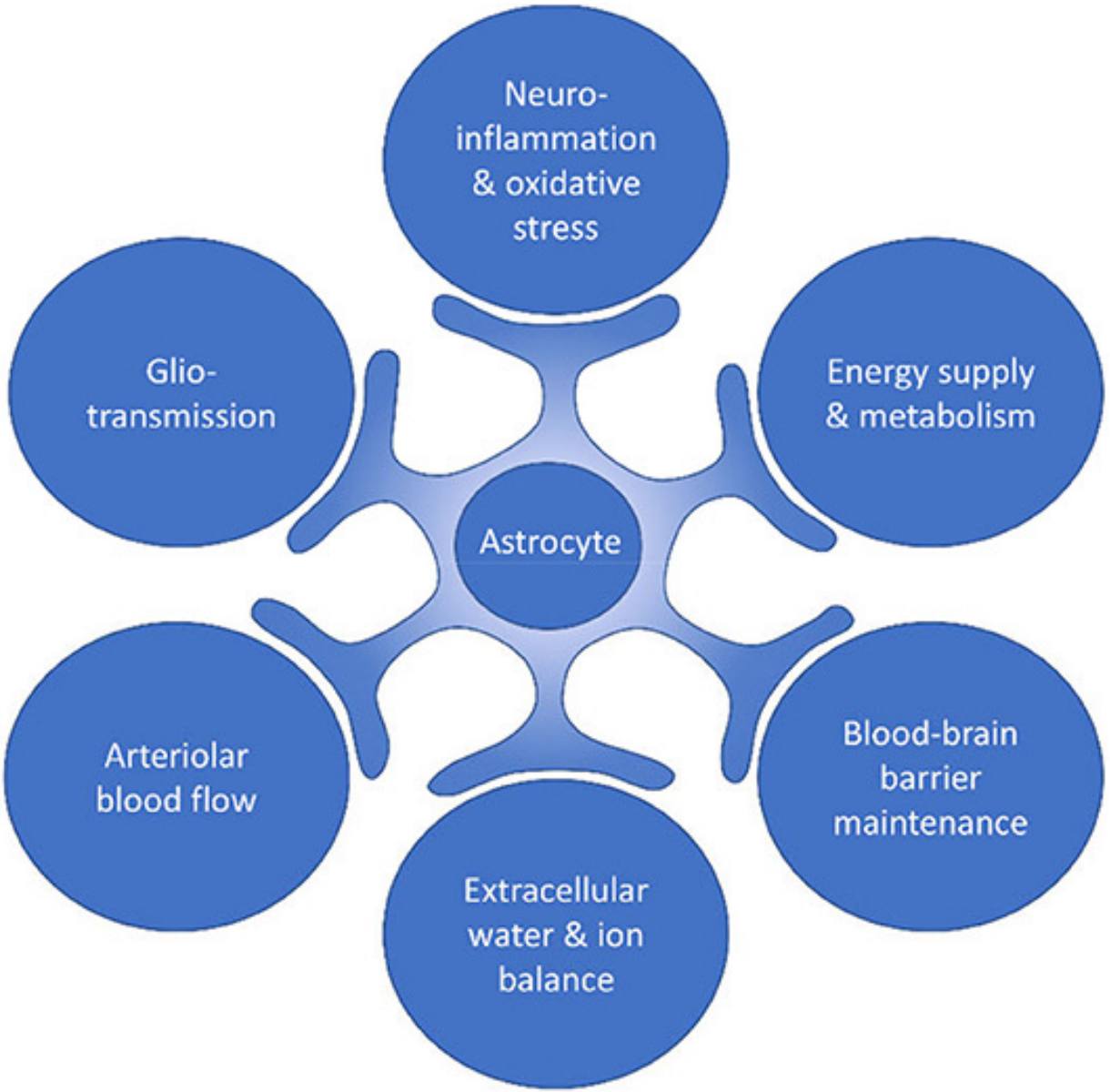


Morphological overview of isolated mixed cortical cells and pure astrocyte culture at different timepoints after isolation. A) 1 day after plating of mixed cortical cells. First astrocytes are attached to the bottom of the flask (black arrows) and dying neurons are in the supernatant. B) 3 days after 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.

Debriefing on Astrocytic functions



Just remind that….

Human astrocytes are larger…….

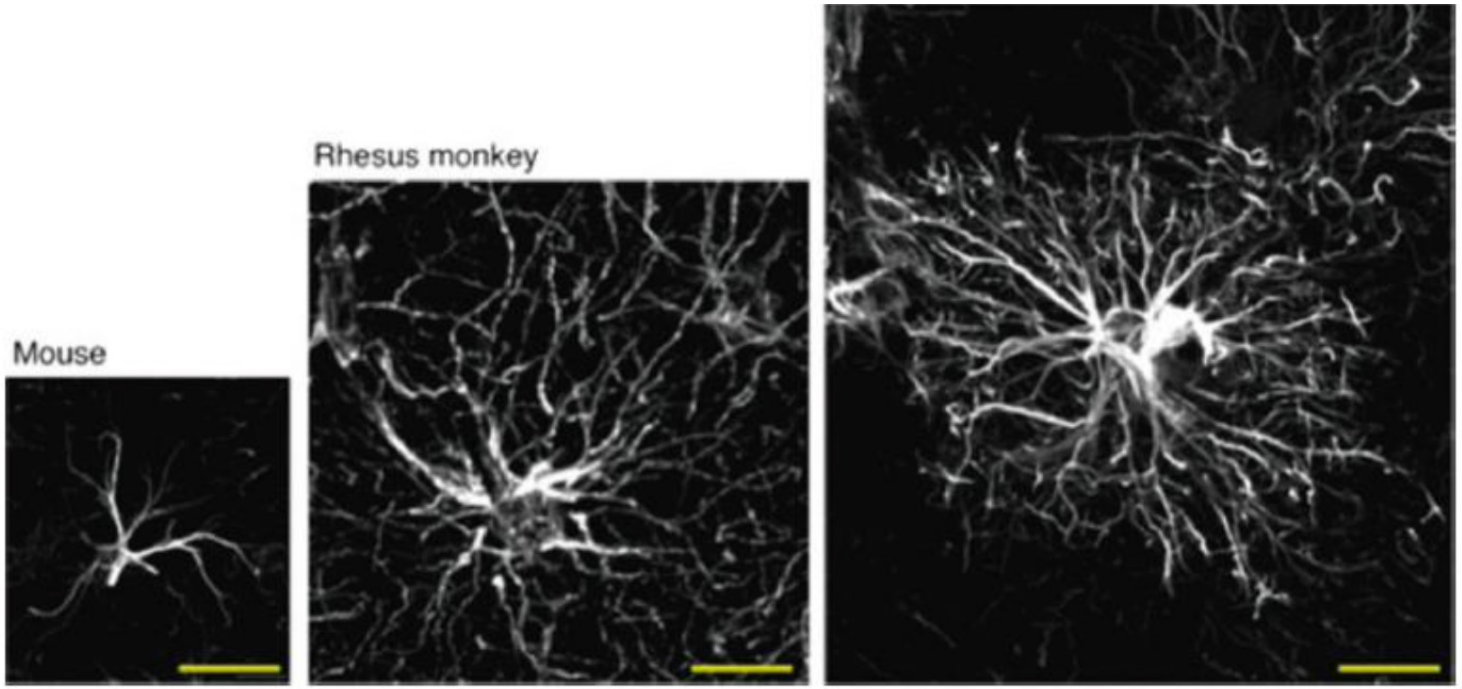


Fig. 6.
Human astrocytes are larger and more complex than rodent and other primates. Mouse, Rhesus Monkey, and Human astrocytes are compared by GFAP staining (*white*). Scale = 20 μm.

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

Human Astrocytes

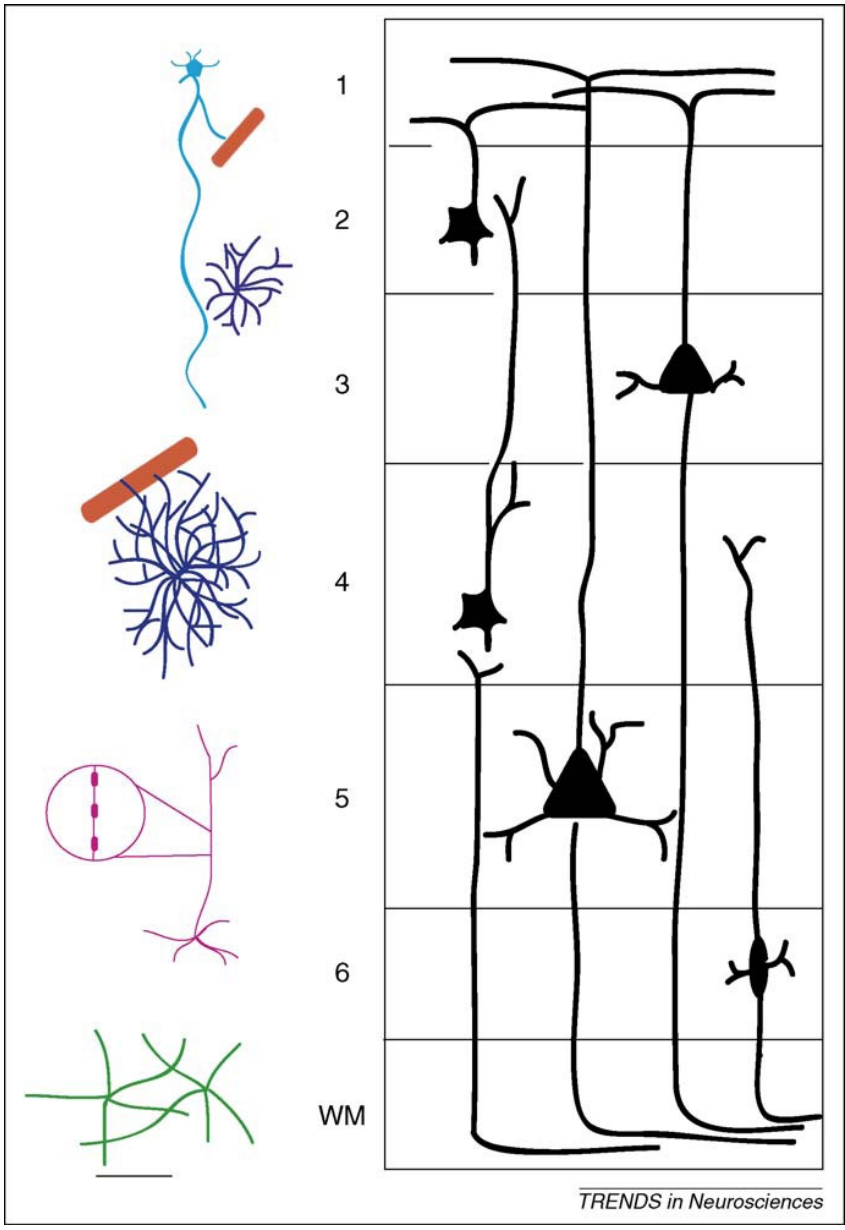
Distinct classes of human astrocytes are located within different layers of the cortex.

Interlaminar astrocytes (light blue) layer 1; send long fibers terminating in layers 3 and 4.

Protoplasmic astrocytes (dark blue) layers 2–6, varable size. Organized in domains associated with neurons and blood vessels.

Polarized astrocytes (pink) layers 5–6 extend long processes,with varicosities

Fibrous astrocytes (green) white matter are not organized in domains.



FURTHER READING

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