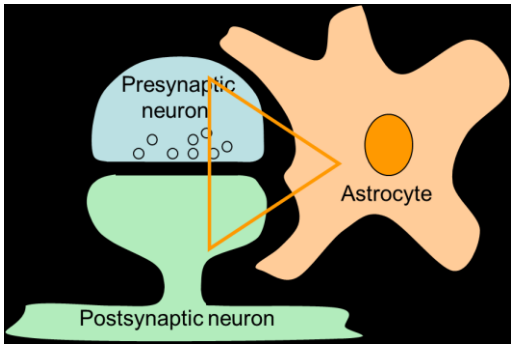
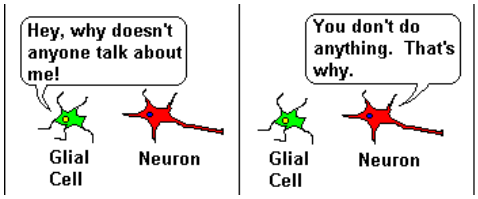
Lesson 4
Astrocytes functions: the Tripartite synapse



History of glia: non-excitable? not exciting?



http://faculty.washington.edu/chudler/glia.html

Term "neuroglia" = "nerve glue" (coined by Virchow in 1846)

First visualized by Santiago Ramon y Cajal and Camillo Golgi (~1900s)

Glial cells are defined as "anything in the brain that is not neurons or blood vessels" and include astrocytes, oligodendrocytes and microglia

Possible reasons for neglecting glial cells:

1) Morphology: lack of long axon-like processes
2) Electrophysiology: lack of mechanism for action potential generation

*"What is the function of glial cells in neuronal centers? … It may remain unresolved for many years to come until physiologists find **direct methods** to attack it". – S. Ramon Y Cajal (1911)*

Functions of astrocytes in the CNS

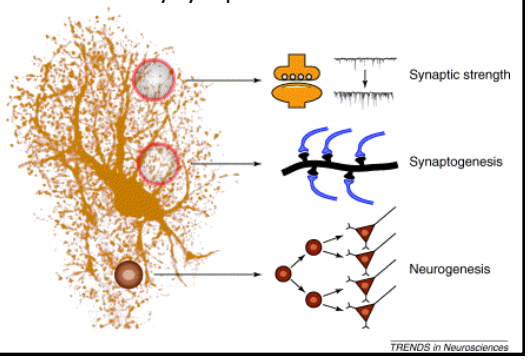
Historically recognized:

- Sequestration of K^+ during neural activity
- Removal of glutamate and GABA at synapses
- Synthesis of precursor for glutamate and GABA
- Neuronal pathfinding
- Blood–brain barrier regulation
- Regulation of extracellular pH

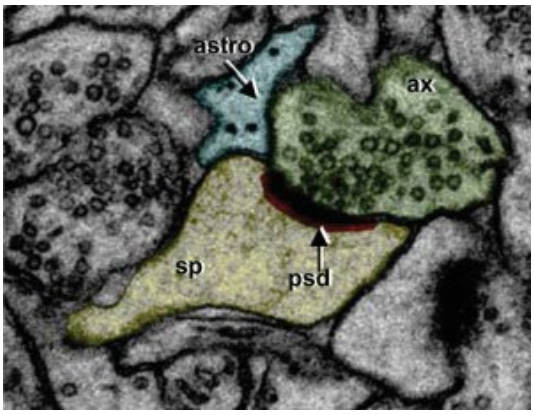
Newly established and emerging:

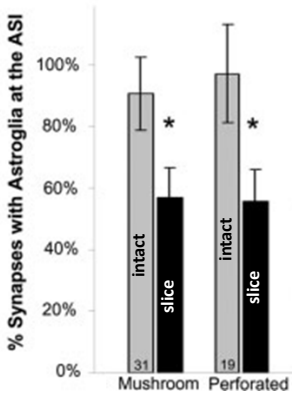
Regulation of neurogenesis in adult brain (e.g. hippocampus)

- Regulation of synaptogenesis
- Modulation of excitatory and inhibitory synapses



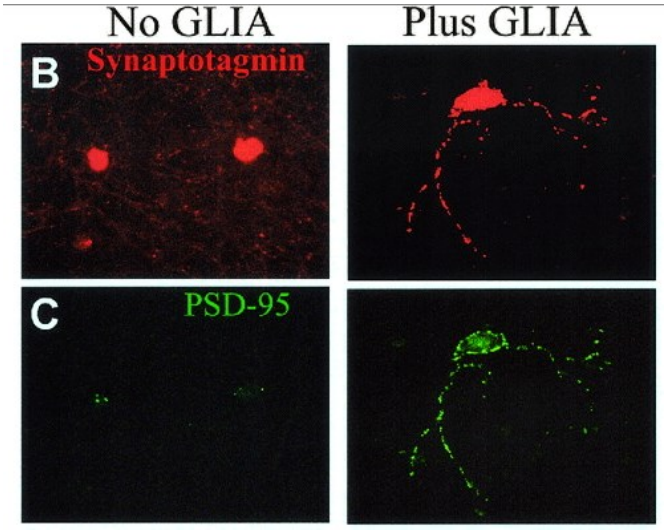
Major portion of synapses has glial component





Witcher et al., *Glia*,2007

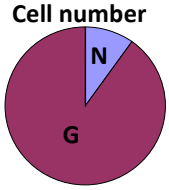
Astrocytes control synapse formation



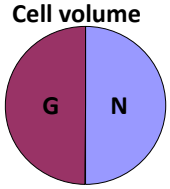
Control of Synapse Number by Glia
Erik M. Ullian, Stephanie K. Sapperstein, Karen S. Christopherson, and Ben A. **Barres**

More glia than neurons in the cortex

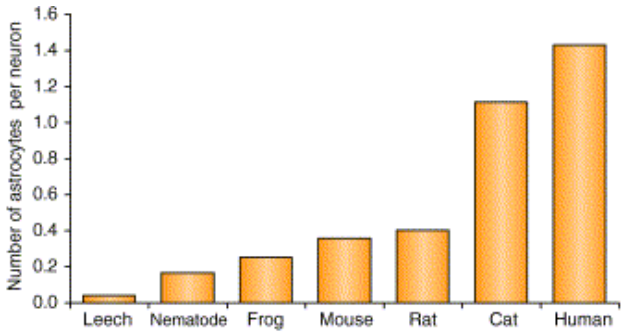
Cell number



Cell volume

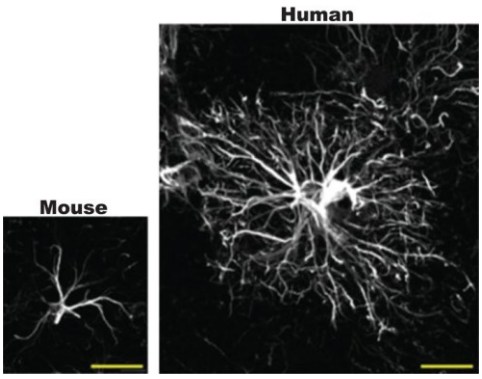


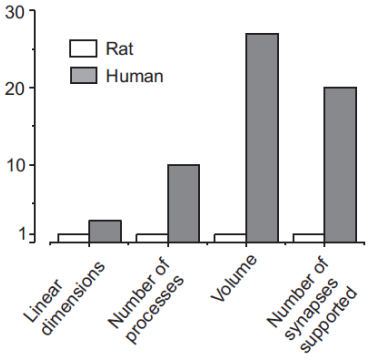
Number of astrocytes per neuron



A curious observation:
Albert Einstein had more glia cells per neuron than an average person.
Diamond et al (1985) Experimental Neurology (vol. 88, pages 198-204)
http://paulcooijmans.lunarpages.com/intro.html

Evolution of astroglia





Verkhratsky & Butt, *Glial Physiology and Pathophysiology*, 2013
http://www.urmc.rochester.edu/labs/Nedergaard-Lab/projects/evolutionary_changes_of_astrocytes

Astrocytes processes fill a volume that is best defined as a polyhedron or a sphere with less than 5% overlap with the volume occupied by adjacent astrocytes

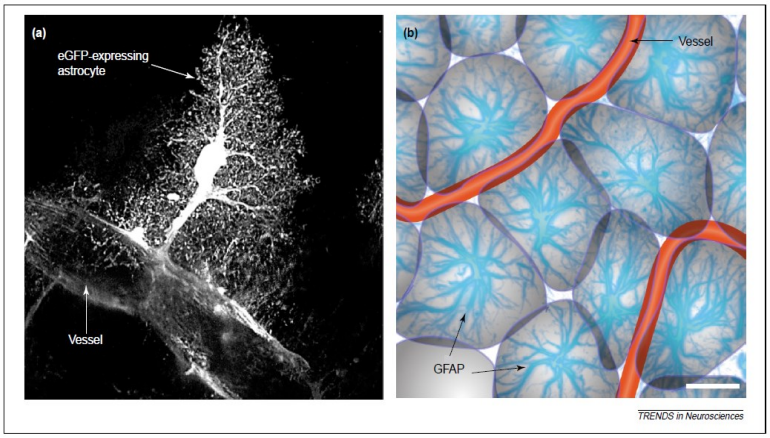
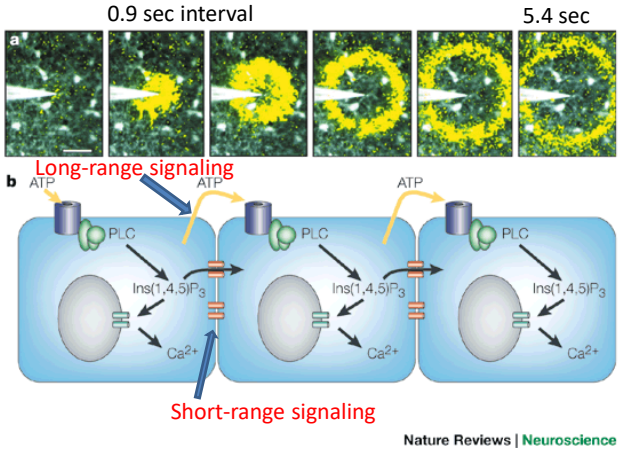


Figure 3. Contact spacing dictates the spatial organization of astrocytes. Astrocytes are homogeneously distributed within the gray matter [11,12]. Their processes are not merely tubular but veil-like and extremely complex. The processes of a single astrocyte densely invest a 3D space of polyhedral shape. **(a)** Two-photon confocal imaging of an enhanced green-fluorescent protein (eGFP)-expressing astrocyte in a cortical slice, illustrating the dense array of processes from a single cell. The misconception that astrocytes are simple star-shaped cells stems from traditional studies using silver impregnation techniques that stained intermediate filaments, and from later studies using antibodies directed against the principal glial filament, glial fibrillary acidic protein (GFAP). GFAP is densely expressed within the cell body and in the primary and secondary processes of astrocytes; these filaments fill a small fraction of the cell and extend into only the larger processes. The 'true' morphology of gray matter astrocytes is revealed with single-cell dye fills or eGFP expression, as shown here. The fine GFAP-negative processes might include the majority of the total volume of the astrocyte, and define in their extent its territorial domain (T. Takano and M. Nedergaard, unpublished). **(b)** Astrocytes are organized in rows along the vessels, which are typically positioned in the narrow interface between astrocytes. This schematic is based on a cortical rat section immunolabeled for GFAP (blue). The astrocytic territories are arbitrarily outlined, demonstrating that the GFAP-positive processes fill only the center of each territory. Vessels are indicated in red.

http://tins.trends.com

Astrocytes "talk" by Ca^{2+} waves

Mechanical stimulation of a retina glial cell



Astrocytes possess a special type of excitability which can be termed **"Ca^{2+} excitability"**. To understand the complex "glial language", one needs **imaging** methods which have been developed relatively recently.

Atrocytes respond with **Ca^{2+} waves** to histamine, noradrenaline, acetylcholine, ATP and GABA

Models of the mechanism of astrocytic Ca2+ signaling

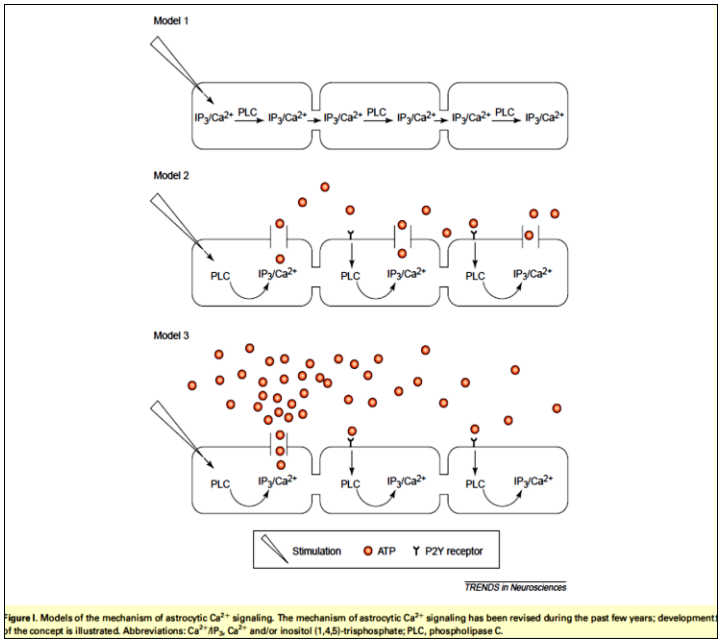


Figure I. Models of the mechanism of astrocytic Ca^{2+} signaling. The mechanism of astrocytic Ca^{2+} signaling has been revised during the past few years; development of the concept is illustrated. Abbreviations: Ca^{2+}/IP_3, Ca^{2+} and/or inositol (1,4,5)-trisphosphate; PLC, phospholipase C.

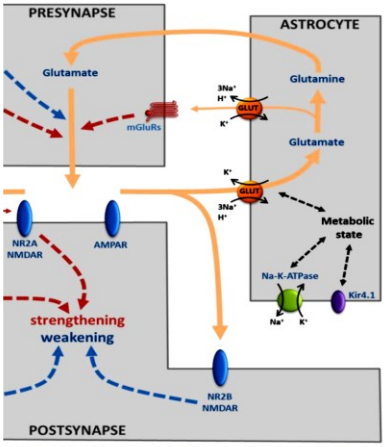
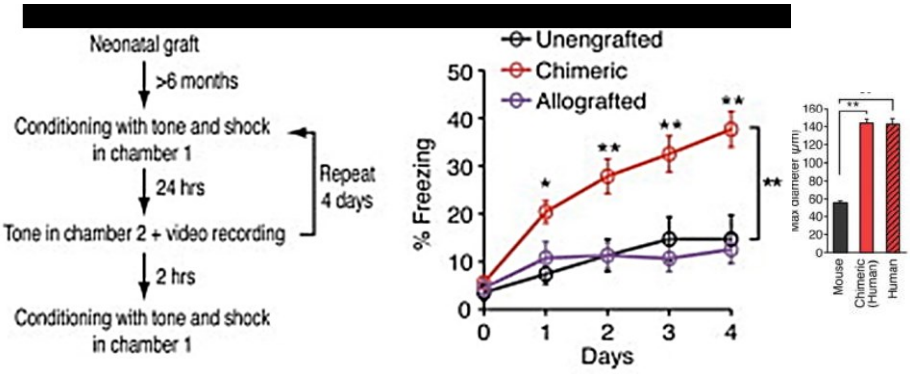


Figure 2. Neuron-astrocyte signaling in the excitatory multipartite synapse. Glutamate-glutamine cycle shown in orange has several key steps: after release from a presynaptic terminal glutamate can activate postsynaptic receptors mGluRs at the astrocytic endfeet or extrasynaptic receptors. Glutamate scavenging kinetics by astrocytic GLUT as well as spatial positioning of PAPs determines both postsynaptic and astrocytic glutamate action. After been scavenged by astrocyte glutamate can be excreted by the reverse uptake or can be sent back to the presynaptic terminal in a form of glutamine. Glutamate metabolism due to its involvement in TCA cycle is tightly related to the metabolic state of the cell and maintenance of ion homeostasis via Na-K-ATPase and potassium channels. Signaling cascades involving cAMP and Ca^{2+} in the astrocyte can influence synaptic transmission in bidirectional fashion, causing presynaptic inhibition or activation via different types of metabotropic purinoreceptors or facilitate postsynaptic LTP via purines and D-serine release. Via releasing or scavenging glycine through the GlyT astrocytes are also involved in presynaptic glycine actions.

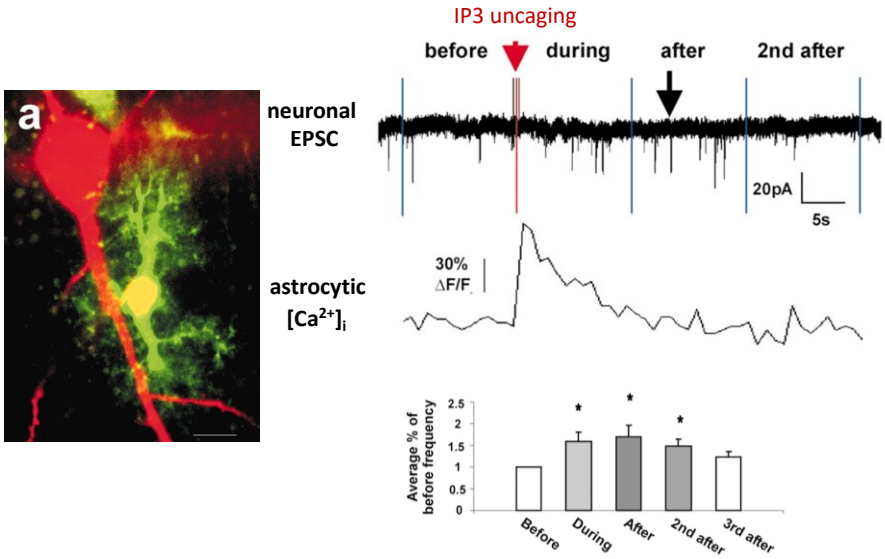
Human glial cells can support learning



Auditory fear conditioning assessed in a cohort of human chimeric, mouse allografted, and unengrafted control mice. Chimeric mice exhibit prolonged freezing behavior in test chamber 2, during exposure to the tonal conditioned stimulus when compared to unengrafted mice and allografted mice. Human astrocytes have larger diameter than mouse astrocytes.

Han et al., *Cell Stem Cell*, 2013

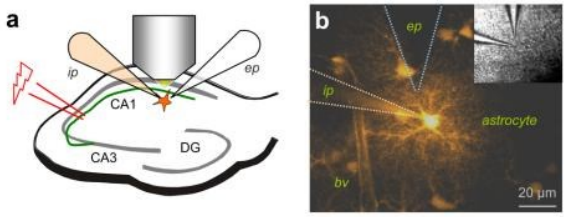
Astrocytes scale neuronal activity



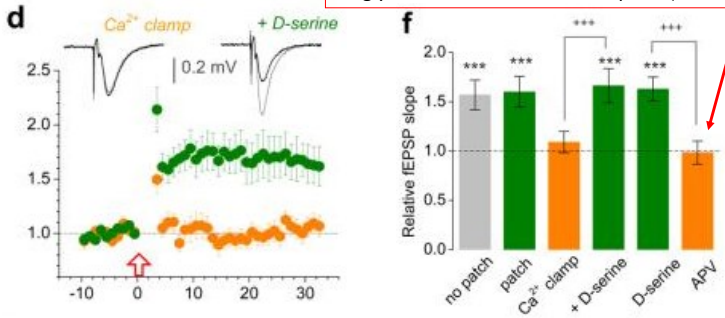
Fiacco & McCarthy, *J. Neurosci.*, 2004

Two examples of astrocytes regulation of synaptic activity

D-serine assists synaptic function



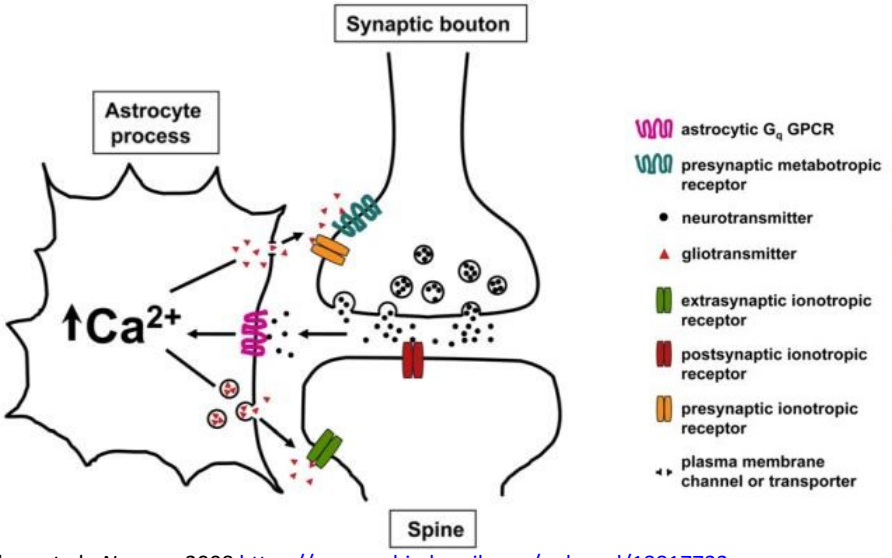
Astrocytes can release D-Serine which binds to the glycine site of Glutamate receptors (NMDA-type)



Henneberger et al., *Nature*, 2010

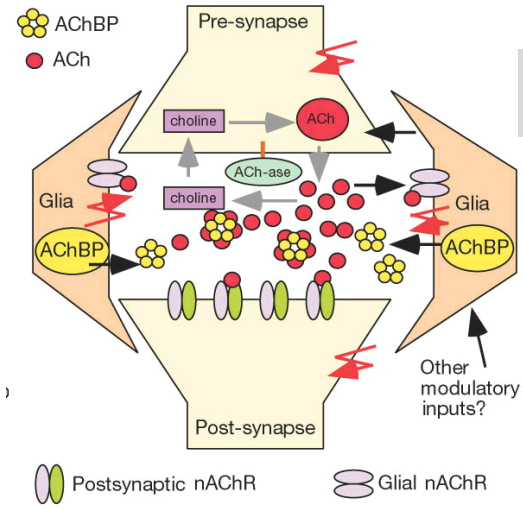
Astrocytes modulate synaptic transmission by releasing glutamate with depressive effect*

(* on amplitude of synaptic activity)



Agulhon et al., *Neuron*, 2008 https://www.ncbi.nlm.nih.gov/pubmed/18817732

Glia works in unspected ways



AChBP as a nAChR decoy

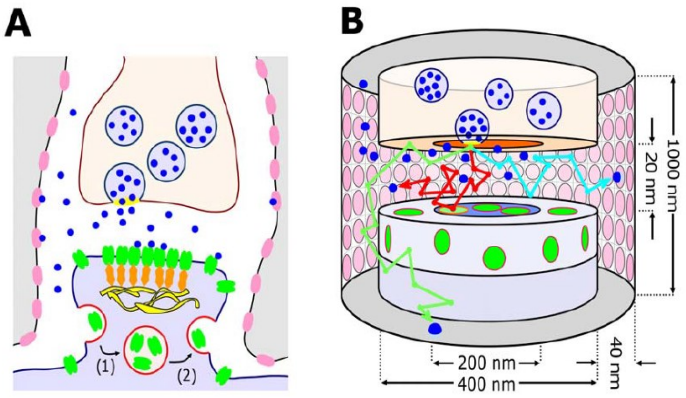
Figure 8 Model of the role of AChBP in neurotransmission. A basal level of AChBP is present in the synaptic cleft. Presynaptic ACh release can lead to activation of postsynaptic receptors and to EPSPs. In parallel, nAChRs on glia are activated, causing increased release of AChBP into the synapse, which leads to suppression of cholinergic transmission (see Discussion).

Nature 411, 261 - 268 (2001)

How did they figure this out?
(or, what does it take to get a paper in Nature?)

- Observation:
  - Cultured neurons formed Ach synapses
  - When glia were allowed to contact synapses, synaptic depression was observed
- Question:
  - How do the glia **sense** and **respond** to the Ach to modulate transmission?
  - **Sense** ACh: Glia have nAChR, which senses increased Ach and induces release of AChBP from glia into the cleft,
  - **Respond**: AchBP (identified by Bungarotoxin purification scheme, partial AA sequence)suppressing transmission (negative feedback).

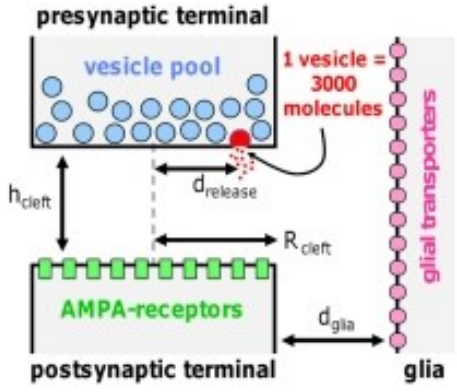
Representation of the synapse dynamics at the tripartite synapse

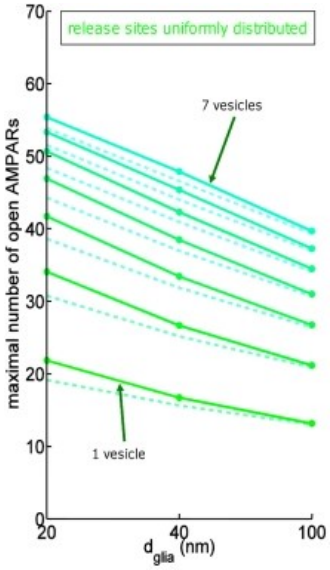


(A) The synapse is surrounded by astroglial processes containing glutamate transporters (GLTs). Released glutamate (blue) diffuses in the cleft and binds to AMPARs (green) or GLTs (pink). AMPARs diffuse between the PSD, where they can attach to scaffolding molecules (orange) and the extrasynaptic regions, where they can undergo endocytosis (1) and exocytosis (2), maintaining the number of AMPARs at the post-synaptic terminal. (B) Two co-axial cylinders represent the pre- and postsynaptic terminal, forming a gap which represents the synaptic cleft. AMPARs (green) are distributed inside and outside the PSD. The trajectory of a glutamate molecule as illustrated by red, blue or green arrows corresponds to binding to AMPARs, GLTs or diffusing away from the cleft (at 500 nm), respectively.

Freche et al., *PLoS One*, 2011

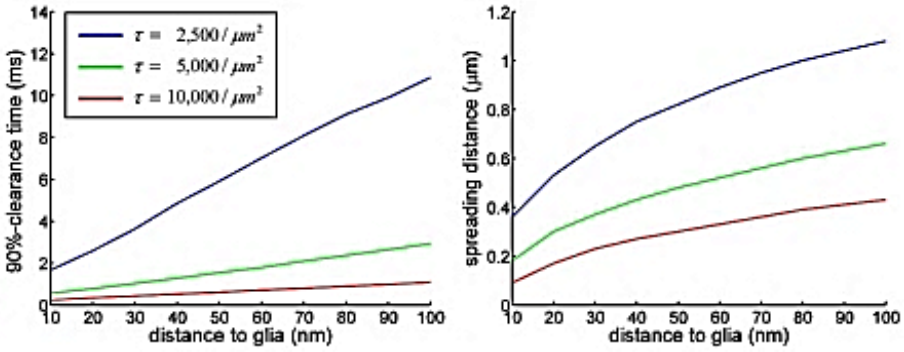
Morphology is a key to synaptic efficacy





Freche et al., *PLoS One*, 2011

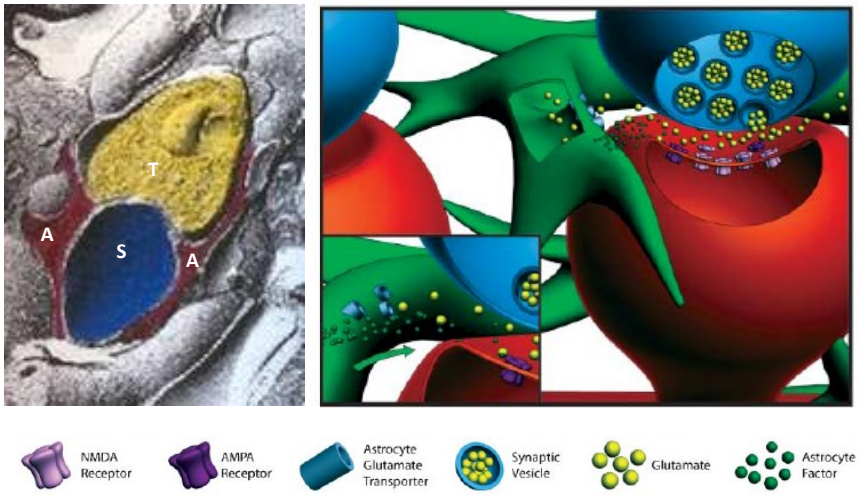
Glial coverage of the synapse is critical for neurotransmitter actions



Clearance time and spreading distance for various glial sheath distances from 10 nm to 100 nm and transporter densities from 2,500 to 5,000 to 10,000=mm$_2$

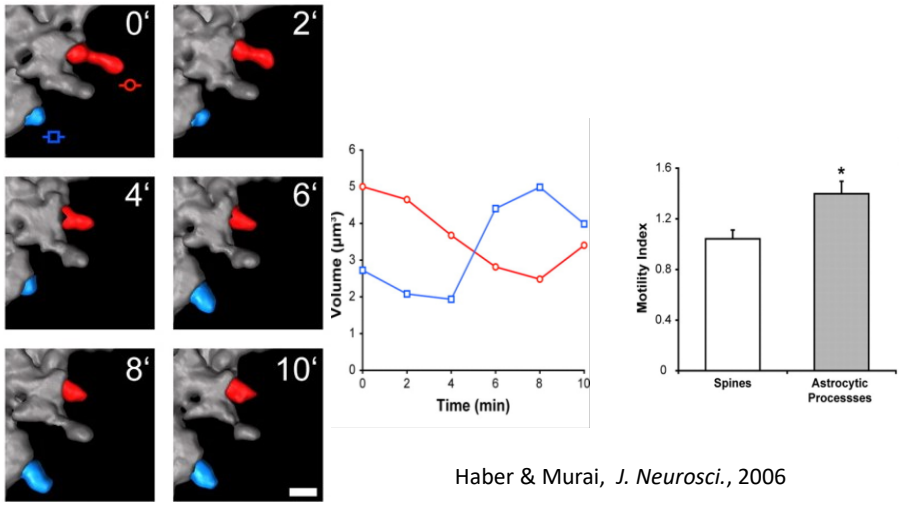
Freche et al., *PLoS One*, 2011

Morphological and functional plasticity

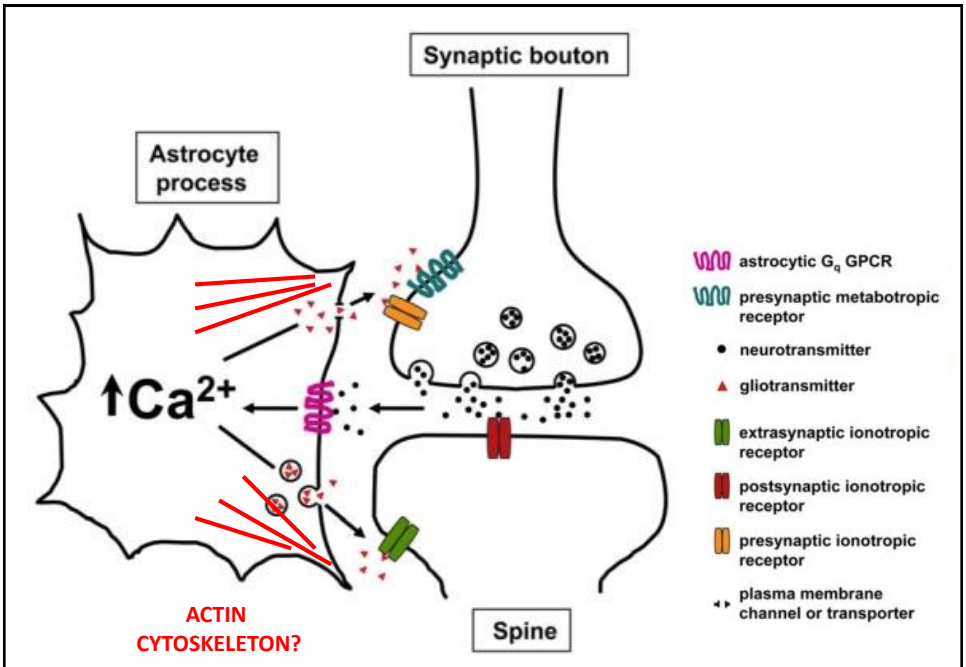


Haber & Murai, *Neuron Glia Biology*, 2006

Astrocytic processes demonstrate high motility rates



Haber & Murai, *J. Neurosci.*, 2006



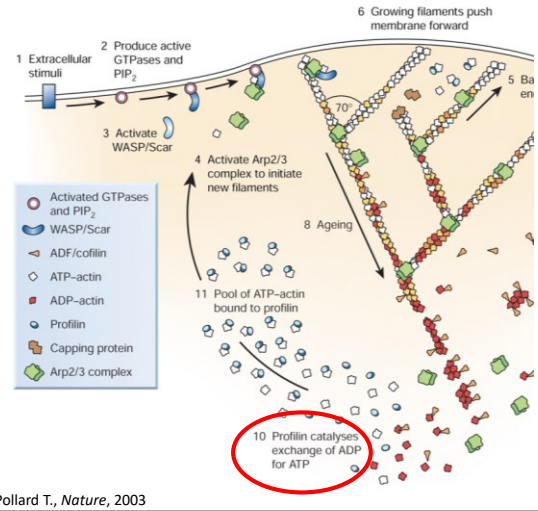
Agulhon et al., *Neuron*, 2008

FEBS Letters 457 (1999) 470–474 FEBS 22531

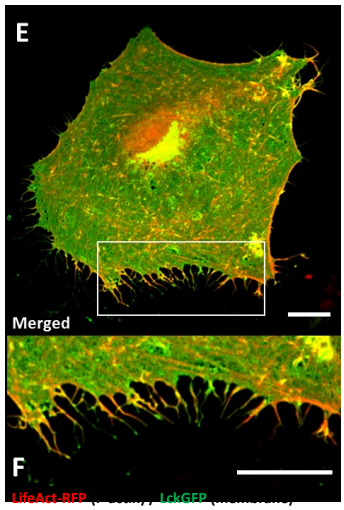
Distinct roles of profilin in cell morphological changes: microspikes, membrane ruffles, stress fibers, and cytokinesis

Shiro Suetsugu, Hiroaki Miki, Tadaomi Takenawa*

Department of Biochemistry, Institute of Medical Science, University of Tokyo, Tokyo, Japan

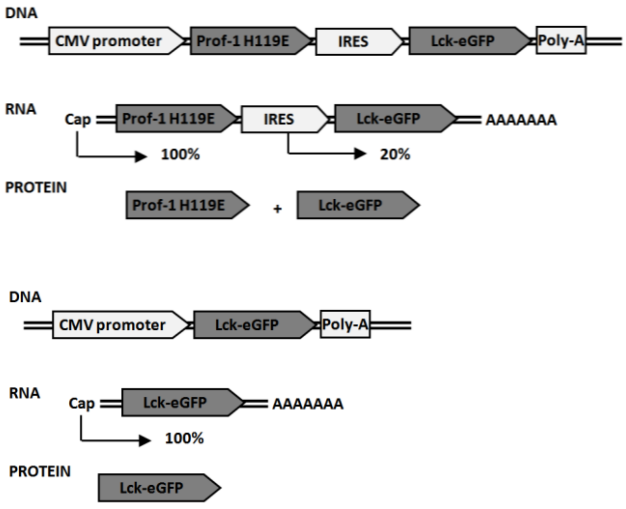


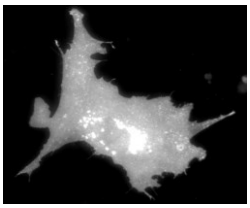
Pollard T., *Nature*, 2003

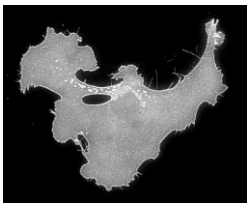


Molotkov et al., *Cell Calcium*, 2013

Bicistronic expression system: simultaneous mutant expression and membrane tracing

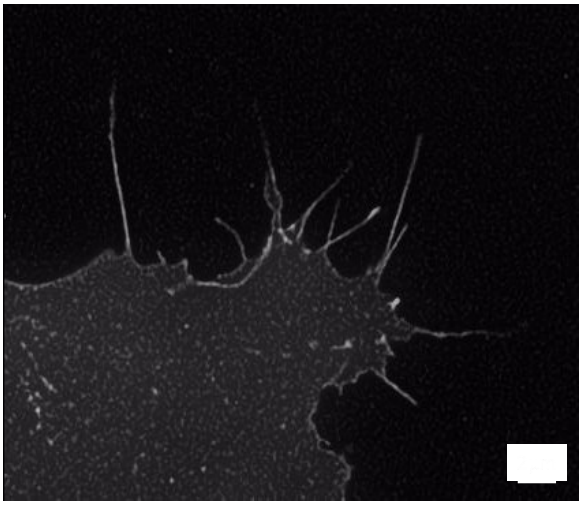


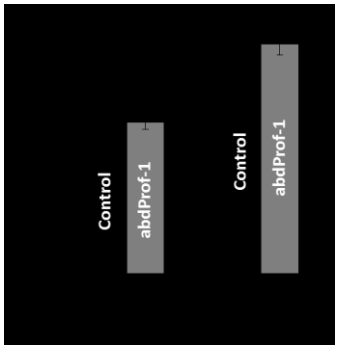




Molotkov et al., *Cell Calcium*, 2013

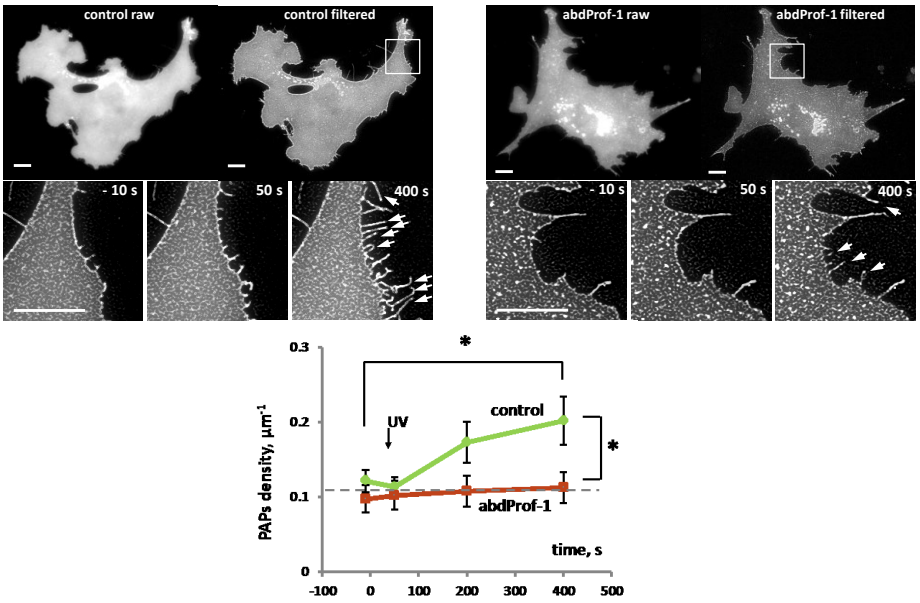
Basal astrocyte motility is not affected by abdProf-1 overexpression





Molotkov et al., *Cell Calcium*, 2013

Peripheral astrocytic processes density after Ca^{2+} uncaging



Molotkov et al., *Cell Calcium*, 2013

CONTROVERSY

2007

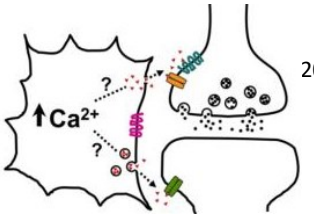
Selective Stimulation of Astrocyte Calcium In Situ Does Not Affect Neuronal Excitatory Synaptic Activity

Todd A. Fiacco,[1,*] Cendra Agulhon,[1] Sarah R. Taves,[1] Jeremy Petravicz,[1] Kristen B. Casper,[1] Xinzhong Dong,[2] Ju Chen,[3] and Ken D. McCarthy[1]

2008

Loss of IP_3 Receptor-Dependent Ca^{2+} Increases in Hippocampal Astrocytes Does Not Affect Baseline CA1 Pyramidal Neuron Synaptic Activity

Jeremy Petravicz,[1] Todd A. Fiacco,[2] and Ken D. McCarthy[1,2]



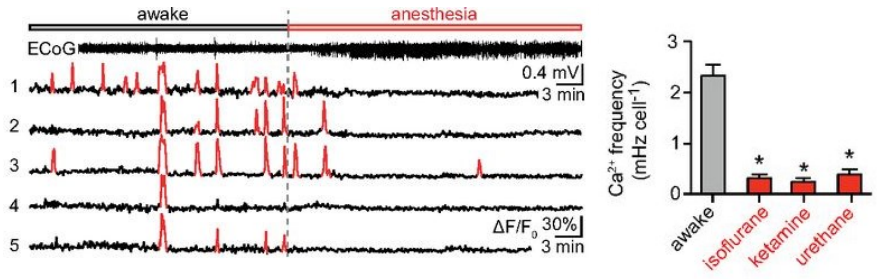
2010

Hippocampal Short- and Long-Term Plasticity Are Not Modulated by Astrocyte Ca^{2+} Signaling

Cendra Agulhon,[1,*] Todd A. Fiacco,[2] Ken D. McCarthy[1]

General anesthesia selectively disrupts astrocyte calcium signaling in the awake mouse cortex

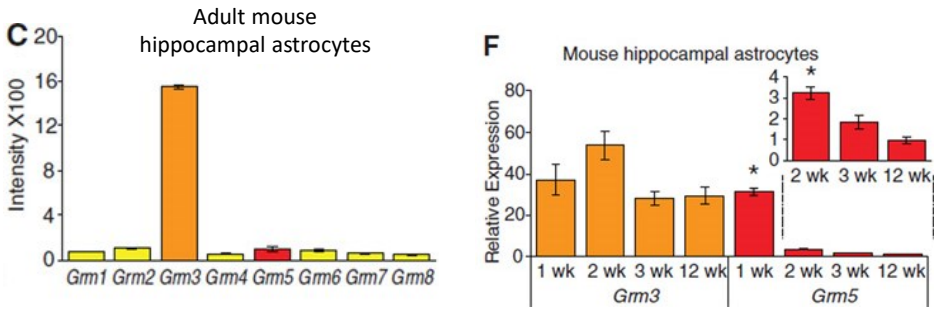
Alexander Stanley Thrane[a,b,c,1,2], Vinita Rangroo Thrane[a,b,c,1], Douglas Zeppenfeld[a], Nanhong Lou[a], Qiwu Xu[a], Erlend Arnulf Nagelhus[b,c], and Maiken Nedergaard[a]



Thrane et al., *PNAS*, 2012

Glutamate-Dependent Neuroglial Calcium Signaling Differs Between Young and Adult Brain

Wei Sun,[1*] Evan McConnell,[1*] Jean-Francois Pare,[2*] Qiwu Xu,[1*] Michael Chen,[1] Weiguo Peng,[1] Ditte Lovatt,[1] Xiaoning Han,[1] Yoland Smith,[2] Maiken Nedergaard[1†]



Sun et al., *Science*, 2013

Conclusions

- Glial cells are able to talk to neurons but their tone depends on situation.
- Morphology and functionality of synapses as well as current internal state of individual synaptic components should be considered in order to understand the role of astrocytes in synaptic function.