MAHSA International Journal of Health and
Medicine, V5, Issue 2; July, 2025
e-ISSN: 2805-5705

Original Article Open Access

THE RESEARCH PROGRESS OF GLIAL CELLS: FUNCTIONAL DYNAMICS IN SYNAPSE FORMATION, MODULATION, AND PLASTICITY

Zhongliang Wei [1,2] Chandramohan Muruganandham[3*] , Chongdong Jian[1,4*]
[1] Department of Anesthesiology, The Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, Guangxi 533000, China.
[2] MAHSA University, Bandar Sauiana Putra, Selangor 42610.Malaysia.
[3] Faculty of Pharmacy and Biomedical Sciences, MAHSA University, SP2, Bandar Saujana Putra, Jenjarom, 42610, Selangor, Malaysia.
[4*]Department of Neurology, The Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, Guangxi 533000, China.

Corresponding Author(s): chandramohan@mahsa.edu.my, jianchongdong@ymun.edu.cn

Received: 10th July 2025 Accepted: 17th July 2025 Published: 30th July 2025

Abstract

Glial cells were once thought to provide only essential physiological support, such as nutrient supply and structural stability for neurons. However, recent research reveals that glial cells play active and complex roles in the nervous system, particularly in synapse formation, modulation of synaptic activity, and cognitive processes like learning and memory. Additionally, their mechanical properties have been found to differ significantly from traditional understanding, emphasizing their dynamic interactions with neurons. This review integrates recent advances in glial cell research, highlighting their contributions to synaptic communication, activity, and signaling in cognitive functions. This article underscores the need to explore further the signaling pathways and molecular mechanisms underlying these processes by synthesizing current findings. Understanding these mechanisms will provide deeper insights into synaptic formation and the regulation of learning and memory.

Keywords: Glial cell, synaptic formation, synaptic activity, learning, memory.

1. Introduction

Glial cells have long been considered mere support cells in the nervous system, providing nutrition and maintaining the extracellular environment. However, emerging research has demonstrated that glial cells play critical roles in synaptic formation, modulation of synaptic activity, and cognitive functions such as learning and memory.

The brain consists of neurons and glial cells, with glial cells comprising 90% of the adult human brain. Despite this large number of glial cells, it has been thought that they only serve as scaffolds, provide nutrients for neurons, remove excessive potassium ions from the synaptic gap, and reabsorb the neurotransmitter glutamate. Pfrieger et al. (1997) established a method for pure neuronal culture. They found that glial cells can significantly promote neuronal synapse formation, influencing the maintenance of synaptic structure and transmission efficacy [1].

In recent years, numerous studies have confirmed that glial cells are not simply supportive and trophic components but also form synaptic connections with neurons. Research has found that each glial cell in the mouse cerebral cortex can contact multiple neuronal bodies, 300–600 dendrites, and about 100,000 synapses from different neurons, integrating feedback information from these neurons [2]. The ratio of glial cells to neurons within the brain varies across different regions and correlates with the size of the individual animal. The increase in the ratio of glial cells may be due to the need to modulate more synapses [3].

There is growing experimental evidence that perisynaptic glial cells also comprise synapses and thus form triple synapses with neuronal pre-synapses and post-synapses (Fig. 1) [4]. Glial cells also change their phenotype during development. Due to this plasticity and their ability to release trophic factors and gliotransmitters, glial cells have complex functions in higher activities of the nervous system, such as learning and memory, and have an essential influence on neurogenesis and synapse formation and maintenance [1]. In addition, glial cells have been shown to communicate information to neurons and synapses actively. The role of glial cells in synaptic plasticity and higher neural activity has been increasingly emphasised.

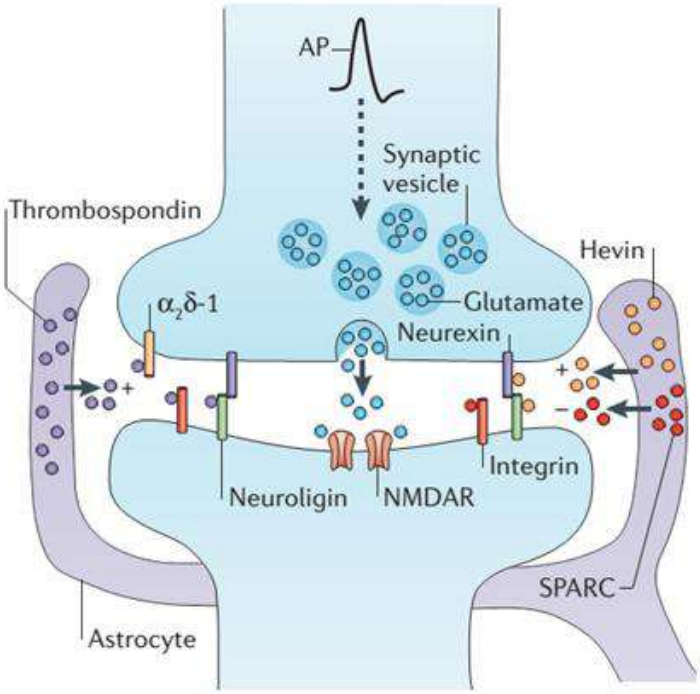

Figure 1: Astrocyte form triple synapses with axon and dendrites [4]

Mechanical properties of glial cells

Rudolf Virchow discovered that there are non-neuronal cells in the brain, which are thought to fill and glue neurons together; hence, they are named glial cells. Regarding mechanical properties, glial cells are considered soft, sticky substances, but not elastic. Another perspective suggests that glial cells directly provide scaffolding and support for neurons. Therefore, they are considered more complex than neurons and an elastic solid substance [5].

For a long time, researchers accepted these two mutually exclusive views. However, with the advent of atomic force microscopy technology, which can determine mechanical properties at the cellular level, the mechanical characterization of neurons and glial cells revealed that the elastic behaviour of both glial cells and neurons is significantly greater than their vicious behaviour. Moreover, surprisingly, glial cells were found to be substantially softer than neurons [5]. These results suggest that glial cells neither support nor glue neurons. Mechanistically, it is hypothesized that glial cells act as a compliant, soft substance that envelops neurons and prevents them from impact damage.

Mechanical stimulation can cause glial cells to generate calcium waves and release ATP [6]. It has been shown that glial cells express force-sensitive ion channels such as Piezo1 and TRPV4, which can transmit the force signals they perceive to neurons [7, 8].

Glial cells and synapse formation

Pfrieger et al. were the first to establish a pure retinal neuron culture method that allowed neurons to survive for weeks without glial cells. Establishing a pure neuronal culture system provides an excellent method for studying the effect of glial cells on synapse formation [1]. Retinal ganglion cells (RGCs) cultured in a medium devoid of glial cells formed only a few synapses. The synapses formed by RGCs were ultrastructurally normal but exhibited very little spontaneous synaptic activity and a high failure rate in synaptic transmission. RGCs co-cultured with glial cells showed a 70-fold and 5-fold increase in the frequency and amplitude of spontaneous excitatory post-synaptic currents (sEPSCs) and a low probability of failure in synaptic transmission [1]. The densities and viability of RGCs were consistent regardless of the presence or absence of glial cells, suggesting that glial cells can promote synapse formation and enhance the efficacy of synaptic transmission.

Further studies have shown that the addition of glia-conditioned medium (GCM) to pure neuronal cultures significantly increased the number of synapses and the frequency and strength of spontaneous excitatory post-synaptic currents of RGCs, even though the RGCs were not in direct contact with the glial cells [1]. In addition to RGCs, glial cells promote synapse formation in hippocampal, cortical, and spinal motor neurons [9, 10]. Glial cells specifically regulate different stages of synapse formation by modulating neuron-specific receptors.

Formation of the exact number and type of synapses is essential for normal neural circuit formation and information transfer. Neural circuit formation occurs in three stages: 1) contact between axons and dendrites or cytosol to form immature synapses; 2) formation of synapses with synaptic transmission efficacy; and 3) removal of excess synapses. Glial cells remove unnecessary synapses and promote synapse formation [11]. The developing brain forms more synapses than are required to maintain regular physiological activity in adulthood, and these excess synapses are gradually removed during development, a process in which glial cells play a direct or indirect role. Gene expression profiling has shown that glial cells express abundant phagocytic receptors, such as Megf10 and Mertk, which have been demonstrated in *Drosophila* to mediate the clearance of synaptic debris by glial cells [12, 13].

Recent studies have also shown that glial cells can indirectly clear synapses. Retinal astrocytes release TGF-β (transforming growth factor β), which induces microglia to express C1q (complement protein C1q) to activate their phagocytosis [14]. Additionally, glial cells can maintain the standard structure of synapses. Six days after removing glial cells from the culture medium, the presynaptic vesicle content (quantal content, QC) and synaptic puncta of RGCs were reduced to 1/4 of their original levels. Conversely, synaptic vesicle protein 2 (SV2) and post-synaptic signature protein (GluR2/3) were significantly reduced to 2.5 times their original levels [15]. These findings suggest that glial cells function by stabilizing and maintaining synapses.

In vivo experiments further demonstrated that glial cells play a crucial role in synapse formation, maintenance, and function during development. One week after birth, the SV2 synaptic depressions in the suprachiasmatic thalamus of the mouse brain were nearly synchronized with the emergence and proliferation of astrocytes. During brain development, most neurons arise before astrocytes and project dendrites and axons to appropriate parts of the brain early [16]. However, most synapses between neurons form once astrocytes are mature. Signals from astrocytes contribute to the increase in synapses in the central nervous system.

Mechanisms of synaptic modulation

GCM significantly increases the average frequency and intensity of neuronal sEPSCs even though glial cells do not come into direct contact with neurons. Studies have shown that soluble factors secreted by glial cells promote synapse formation and efficacy. Mauch et al. used chromatography, two-dimensional electrophoresis, and mass spectrometry to isolate the first glial cell-secreted factor, a lipoprotein formed by the complexation of cholesterol with apolipoprotein E (apoE) [17].. It was found that the addition of cholesterol alone to the culture medium significantly increased the frequency of sEPSC in RGCs, and its effect was similar to that of GCM. Removal of cholesterol from GCM caused a loss of synaptic function, and inhibiting lipoprotein uptake also affected synaptic function. RGCs were cultured at low density for recordings of synaptogenesis in individual neurons, and it was found that cholesterol not only increased the number of auto-synapses (autapses) of RGCs by 8-fold but also enhanced QC by 10-fold and the efficacy of neurotransmitter release [5]. Cholesterol exerts its synapse-promoting effect by improving the production of synapsin and synaptophysin, including synaptic vesicles and presynaptic components. RGCs cultured without GCM can also produce a certain amount of cholesterol but can only maintain neuronal survival, axonal and dendritic differentiation, and the formation of very few immature synapses. Many synapses must be generated from the secretion of large quantities of cholesterol by glial cells and transported by apoE-containing lipoproteins [5]. Recently, it has also been found that glial cells secrete soluble proteins thrombospondins and Hevin that promote excitatory synapse formation [18], and that Wnts, CSPGs (chondroitin sulfate proteoglycans), and TNF-α recruit AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid) receptors into synapses [19, 20]. he results show that TNF-α enhances synaptic efficacy by rapidly increasing the arrival of AMPA receptors to the post-synaptic membrane. Blockade of endogenous TNF-α produces the opposite effect, suggesting that maintaining excitatory synaptic function requires the long-term presence of TNF-α [20]. Since TNF-α can affect the operation of AMPAR (alpha-amino-3-hydroxy-5-methyl-4-isoxazole) receptors (trafficking), any abnormality affecting TNF-α production may lead to alterations in synaptic efficacy during synaptic plasticity formation, such as NMDA (N-methyl-D-aspartic acid) receptor-dependent abnormalities of LTP (long-term potentiation) and LTD (long-term depression). SPARC (Acidic secretory proteins rich in cysteine) inhibits synapse formation while decreasing the entry of AMPAR into the post-synaptic membrane to allow for the formation of a proper number of synapses in the neural network [21, 22]. Neurotrophic factors also activate NMDA receptors [23]. Two receptors involved in Thrombospondin-induced synapse formation have

been identified: (1) α2δ1, previously identified as the auxiliary subunit of voltage-dependent calcium channels, and (2) neuroligin, which has been identified as a molecular chaperone for synaptic proteins [24, 25]. Both receptors are localized at the synaptic site. SPARC regulates synaptic AMPARs through β3-integrins to stabilize them in the synapse [18]. Direct contact between glial cells and neurons is also essential for synapse formation. Local contact between glial cells and neurons increases presynaptic activity, EPSC size, and the number of excitatory synapses. The contact-dependent mechanism is the activation of the neuronal protein kinase C signaling cascade pathway through adhesion molecules and integrin binding [22], and contact can also alter the localization of synaptic adhesion molecules [23].

Glial cells express glutamate transporters, glutamine synthetase, water channels, potassium channels, cell adhesion molecules, and localized transporters, mediating cell-to-cell adhesion and modulating glutamate concentrations in the synaptic gap. Glial cells also express GABA (Gamma-aminobutyric acid), glutamate, endogenous cannabinoid receptors, and synaptic-like microvesicles [24]. Cultured glial cells can respond to neurotransmitters released by neurons by exhibiting elevated intracellular calcium concentrations. Calcium ions can be transmitted between neighboring glial cells in the formation of calcium waves. This suggests that glial cells can respond to neuronal activity and communicate over long distances. Neurotransmitters cause the release of calcium ions from the intracellular IP3-mediated Endo calcium pool by acting on the GPCR (Guanosine-binding Protein Coupled Receptor) [24]. Elevated intracellular calcium concentration induces glial cells to release gliotransmitters, including ATP (adenosine triphosphate), adenosine, glutamate, D-serine, and other gliotransmitters, which can affect neuronal activity. Gliotransmitters directly affect pre- and post-synaptic receptors, affecting synaptic efficacy and plasticity. For example, after the release of ATP from glial cells, ATP is rapidly degraded by extracellular nucleotidase to adenosine [24], which acts either on the presynaptic A1R (Adenosine A1 Receptor) to inhibit the release of presynaptic vesicles or on the post-synaptic A2R (Adenosine A2 Receptor) to enhance synaptic strength. The NMDAR (N-methyl-D-aspartate receptor) co-agonist D-serine released by glial cells modulates receptor activity and affects synaptic plasticity. Glutamate release acts on metabotropic glutamate receptors and presynaptic NMDAR to modulate synaptic efficacy [24]. Since astrocytes control the diffusion and concentration of extracellular glutamate to regulate synaptic transmission, alterations in glial cell coverage in neurons near synapses may affect glutamate clearance and synaptic transmission [25-27]. The glutamate transporter GLT-1 expressed in glial cells regulates the concentration and diffusion of extracellular glutamate, activates presynaptic metabotropic glutamate receptors, inhibits glutamate release, and thus exerts a modulatory effect on glutamatergic neurotransmission [28]. Since glutamate is an essential excitatory neurotransmitter in the central nervous system, this role of glial cells may be related to various brain functions such as learning, cognition, and memory. Glial cells communicate with neurons via calcium signaling in brain slices and live animals in addition to culture conditions [29]. Glial cell protrusions are in close contact with synapses due to the close contact with synapses, and this provides a physical barrier that prevents the diffusion of neurotransmitters from the synaptic gap. Morphological studies have shown that glial cell protrusions are in contact with synapses, leading to more stable synapses. Real-time imaging techniques show a dynamic and active process of constant extension and retraction of glial cell protrusions [29].

Glial cells in synaptic plasticity

Various glial cell-secreted factors have been identified to regulate excitatory synapse formation. Although there has been evidence that glial cells promote GABAergic and glycinergic inhibitory synapse formation, the relevant glial cell-secreted factors have not been identified, and the molecular mechanisms are unclear [30, 31]. Identifying

signaling molecules that regulate inhibitory synapse formation will help elucidate how glial cells regulate the balance between excitatory and inhibitory synapses during brain development, which is essential for understanding and treating epilepsy and autism. The same glial factors have opposite or different effects on forming excitatory and inhibitory synapses. For example, thrombospondin promotes excitatory synapse formation and does not affect inhibitory synapse formation [30]. TNF increases membrane expression levels of AMPAR but decreases membrane expression levels of GABAAR, increasing neuronal excitability [31]. Thrombospondin decreases mature synaptic AMPAR levels and increases glycine receptor membrane expression, leading to a decrease in neuronal excitability, which is opposite to the effect of TNFα [32, 33], consistent with the report that thrombospondin induces the formation of structural synapses with post-synaptic silencing [18]. Thrombospondin induces non-functional synapse formation, and glypicans and CSPGs are responsible for recruiting synaptic AMPAR levels to regulate synaptic strength [34-37].

Outlook

Over the past decade, much evidence has shown that glial cells play an important regulatory role at each stage of synapse formation, maturation, clearance, and maintenance. The signaling pathways through which glial cells regulate synapse development and plasticity are an area for future research. Further understanding of how glial cells secrete signaling molecules in response to neuronal activity will help us understand the mechanisms by which glial cells regulate synapse formation and function. Another critical area of research is whether human glial cells share the exact regulatory mechanisms as primate glial cells. Understanding the unique features of human glial cells will provide new insights into the basis of human cognition and offer a molecular foundation for understanding the neurodevelopmental abnormalities that result from glial cell lesions. Although existing research has revealed the multiple roles of glial cells in the nervous system, there are still gaps in knowledge regarding their specific mechanisms in synaptic pruning and neuroplasticity. These findings suggest that glial cells may be involved in the formation and maintenance of synapses through complex signaling networks. Still, the specific molecular mechanisms and cellular processes have not been fully elucidated. Future research must explore how glial cells respond to neuronal activity by secreting signaling molecules and how these processes affect synaptic formation and function.

This section highlights potential research areas, including signaling pathways through which glial cells influence synaptic formation and maintenance. It explores the implications of glial cell research for understanding human cognition and neurodevelopmental disorders.

Conclusion

Glial cells are more than passive participants in the nervous system; they are dynamic regulators of synaptic and cognitive functions. Further research is essential to uncover the molecular underpinnings of their roles in neuroplasticity and brain development.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (grant No. 81860244) and the Guangxi Natural Science Foundation (Grant No. 2018GXNSFAA281051). We thank the National Natural Science Foundation of China (81860244) and the Guangxi Natural Science Foundation for financial support （2018GXNSFAA281051）.

Ethical Approval

This study involving human participants was approved by the Medical Ethics Committee of the Affiliated Hospital of Youjiang Medical University for Nationalities, which conforms to the relevant medical ethics regulations of the state and the affiliated hospital of Youjiang Medical University for Nationalities.

The ethical review number is 2022031406.

Project source: The National Natural Science Foundation of China (81860244).

The Medical Ethics Committee of the Affiliated Hospital of Nationalities

REFERENCES

[1] Pfrieger, F.W. and B.A. Barres, *Synaptic efficacy enhanced by glial cells in vitro.* Science, 1997. **277**(5332): p. 1684-7.

[2] Carlo, C.N. and C.F. Stevens, *Structural uniformity of neocortex, revisited.* Proc Natl Acad Sci U S A, 2013. **110**(4): p. 1488-93.

[3] Verkhratsky, A. and M. Nedergaard, *Physiology of Astroglia.* Physiol Rev, 2018. **98**(1): p. 239-389.

[4] Ostroff, L.E., et al., *Accumulation of Polyribosomes in Dendritic Spine Heads, But Not Bases and Necks, during Memory Consolidation Depends on Cap-Dependent Translation Initiation.* J Neurosci, 2017. **37**(7): p. 1862-1872.

[5] Navneet, S., K. Wilson, and B. Rohrer, *Müller Glial Cells in the Macula: Their Activation and Cell-Cell Interactions in Age-Related Macular Degeneration.* Invest Ophthalmol Vis Sci, 2024. **65**(2): p. 42.

[6] Maneshi, M.M., F. Sachs, and S.Z. Hua, *Heterogeneous Cytoskeletal Force Distribution Delineates the Onset Ca(2+) Influx Under Fluid Shear Stress in Astrocytes.* Front Cell Neurosci, 2018. **12**: p. 69.

[7] Velasco-Estevez, M., et al., *Piezo1 regulates calcium oscillations and cytokine release from astrocytes.* Glia, 2020. **68**(1): p. 145-160.

[8] Shi, M., et al., *Glial cell-expressed mechanosensitive channel TRPV4 mediates infrasound-induced neuronal impairment.* Acta Neuropathol, 2013. **126**(5): p. 725-39.

[9] Xu, J., N. Xiao, and J. Xia, *Thrombospondin 1 accelerates synaptogenesis in hippocampal neurons through neuroligin 1.* Nat Neurosci, 2010. **13**(1): p. 22-4.

[10] Diniz, L.P., et al., *Astrocyte-induced synaptogenesis is mediated by transforming growth factor β signaling through modulation of D-serine levels in cerebral cortex neurons.* J Biol Chem, 2012. **287**(49): p. 41432-45.

[11] Clarke, L.E. and B.A. Barres, *Emerging roles of astrocytes in neural circuit development.* Nat Rev Neurosci, 2013. **14**(5): p. 311-21.

[12] Liddelow, S.A., et al., *Neurotoxic reactive astrocytes are induced by activated microglia.* Nature, 2017. **541**(7638): p. 481-487.

[13] Hilu-Dadia, R., et al., *Draper-mediated JNK signaling is required for glial phagocytosis of apoptotic neurons during Drosophila metamorphosis.* Glia, 2018. **66**(7): p. 1520-1532.

[14] Berkowitz, S., et al., *Complement and Coagulation System Crosstalk in Synaptic and Neural Conduction in the Central and Peripheral Nervous Systems.* Biomedicines, 2021. **9**(12).

[15] Heir, R., et al., *Astrocytes Are the Source of TNF Mediating Homeostatic Synaptic Plasticity.* J Neurosci, 2024. **44**(14).

[16] Xie, Y., et al., *Astrocyte-neuron crosstalk through Hedgehog signaling mediates cortical synapse development.* Cell Rep, 2022. **38**(8): p. 110416.

[17] Vivi, E. and B. Di Benedetto, *Brain stars take the lead during critical periods of early postnatal brain development: relevance of astrocytes in health and mental disorders.* Mol Psychiatry, 2024.

[18] Jones, E.V. and D.S. Bouvier, *Astrocyte-secreted matricellular proteins in CNS remodelling during development and disease.* Neural Plast, 2014. **2014**: p. 321209.

[19] Allen, N.J., *Role of glia in developmental synapse formation.* Curr Opin Neurobiol, 2013. **23**(6): p. 1027-33.

[20] Kleidonas, D., et al., *Microglia modulate TNFα-mediated synaptic plasticity.* Glia, 2023. **71**(9): p. 2117-2136.

[21] Jones, E.V., et al., *Astrocytes control glutamate receptor levels at developing synapses through SPARC-beta-integrin interactions.* J Neurosci, 2011. **31**(11): p. 4154-65.

[22] Kucukdereli, H., et al., *Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC.* Proc Natl Acad Sci U S A, 2011. **108**(32): p. E440-9.

[23] Zhang, X., et al., *NG2 glia-derived GABA release tunes inhibitory synapses and contributes to stress-induced anxiety.* Nat Commun, 2021. **12**(1): p. 5740.

[24] Lau, L.A., et al., *α2δ-1 Signaling Drives Cell Death, Synaptogenesis, Circuit Reorganization, and Gabapentin-Mediated Neuroprotection in a Model of Insult-Induced Cortical Malformation.* eNeuro, 2017. **4**(5).

[25] Choi, J.G., et al., *Inhibition of angiotensin converting enzyme increases PKCβI isoform expression via activation of substance P and bradykinin receptors in cultured astrocytes of mice.* J Vet Sci, 2023. **24**(2): p. e26.

[26] Elmariah, S.B., et al., *Astrocytes regulate inhibitory synapse formation via Trk-mediated modulation of postsynaptic GABAA receptors.* J Neurosci, 2005. **25**(14): p. 3638-50.
[27] Agulhon, C., et al., *What is the role of astrocyte calcium in neurophysiology?* Neuron, 2008. **59**(6): p. 932-46.
[28] Thrane, A.S., et al., *General anesthesia selectively disrupts astrocyte calcium signaling in the awake mouse cortex.* Proc Natl Acad Sci U S A, 2012. **109**(46): p. 18974-9.
[29] Chever, O., et al., *Astroglial connexin 43 sustains glutamatergic synaptic efficacy.* Philos Trans R Soc Lond B Biol Sci, 2014. **369**(1654): p. 20130596.
[30] Danbolt, N.C., *Glutamate uptake.* Prog Neurobiol, 2001. **65**(1): p. 1-105.
[31] Nishida, H. and S. Okabe, *Direct astrocytic contacts regulate local maturation of dendritic spines.* J Neurosci, 2007. **27**(2): p. 331-40.
[32] Stellwagen, D., et al., *Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha.* J Neurosci, 2005. **25**(12): p. 3219-28.
[33] Hughes, E.G., S.B. Elmariah, and R.J. Balice-Gordon, *Astrocyte secreted proteins selectively increase hippocampal GABAergic axon length, branching, and synaptogenesis.* Mol Cell Neurosci, 2010. **43**(1): p. 136-45.
[34] Allen, N.J., et al., *Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors.* Nature, 2012. **486**(7403): p. 410-4.
[35] Lee, E. and W.S. Chung, *Glial Control of Synapse Number in Healthy and Diseased Brain.* Front Cell Neurosci, 2019. **13**: p. 42.
[36] Baldwin, K.T. and C. Eroglu, *Astrocytes "Chordinate" Synapse Maturation and Plasticity.* Neuron, 2018. **100**(5): p. 1010-1012.
[37] Accorsi-Mendonça, D., L.G.H. Bonagamba, and B.H. Machado, *Astrocytic modulation of glutamatergic synaptic transmission is reduced in NTS of rats submitted to short-term sustained hypoxia.* J Neurophysiol, 2019. **121**(5): p. 1822-1830.