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Glioactive ATP controls BDNF recycling in cortical astrocytes

Beatrice Vignoli^a and Marco Canossa^{a,b}

^aCentre for Integrative Biology (CIBIO), University of Trento, Povo (TN), Italy; ^bEuropean Brain Research Institute (EBRI)—Rita Levi-Montalcini, Rome, Italy

ABSTRACT

We have recently reported that long-term memory retention requires synaptic glia for proBDNF uptake and recycling. Through the recycling course, glial cells release endocytic BDNF, a mechanism that is activated in response to glutamate *via* AMPA and mGluR/II receptors. Cortical astrocytes express receptors for many different transmitters suggesting for a complex signaling controlling endocytic BDNF secretion. Here, we demonstrated that the extracellular nucleotide ATP, activating P2X and P2Y receptors, regulates endocytic BDNF secretion in cultured astrocytes. Our data indicate that distinct glioactive molecules can participate in BDNF glial recycling and suggest that cortical astrocytes contributing to neuronal plasticity can be influenced by neurotransmitters in tune with synaptic needs.

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Most studies focusing on astrocyte-synapse interaction point at identifying specific molecules and signaling pathways that unveils the impact of astrocytes on synaptic transmission and plasticity. In a recent publication, we provided clear evidence that long-term potentiation (LTP) requires synaptic glia for (pro)-brain-derived neurotrophic factor (proBDNF) clearance and subsequent recycling (Fig. 1).¹ The neurotrophin-uptake receptor p75^{NTR} is expressed on astrocytes,² that clear excessive proBDNF released at the synapse following LTP-inducing electrical stimulation.³ Endocytic proBDNF is then released as mature protein, which provides a source of neurotrophin that is essential to sustain TrkB phosphorylation in post-synaptic neurons.⁴⁻⁸ Given BDNF glial recycling, neurotrophin signaling is preserved for both strengthening synaptic activity and consolidating memory.

Astrocytes are not electrically excitable cells; thus, receiving signal from neurons at synaptic cleft is the most expected mechanism for these cells to regulate BDNF recycling. This provides a model by which astrocytes, that express receptors sensing transmitter release from active synapses, transduce internal signals leading to endocytic BDNF secretion. Astrocytes express receptors for many different transmitters and modulators, including glutamate, ATP, adenosine, and guanosine and display calcium signaling in response to their stimulation.⁹ This raises the possibility that BDNF glial recycling is highly regulated, and

suggests that complex activation of transmitter receptors on glial cells controls final neurotrophin availability. This idea, which proposes a novel mechanism in neuron-glia interaction, suggests that astrocytes are functional targets for multiple transmitters and they can respond to and, in turn, regulate plasticity in tune with synaptic needs. In support of this hypothesis, we provided clear evidence that in addition to glutamate the extracellular nucleotide ATP regulates endocytic BDNF secretion in cultured cortical astrocytes.

Recycling of BDNF for transmitter-induced secretion by astrocytes

To obtain insight on the triggering events responsible for endocytic BDNF secretion by astrocytes, we used astrocyte cultures from P1-2 rat cortices, in which basal and stimulated neurotrophin secretion were not detected.³ Astrocytes were incubated with a mixture of precursor and mature BDNF isoforms (mix) for 10 min. This timing was chosen, as it correlates with sufficient proBDNF internalization in these cells.³ Astrocytes were then placed in a perfusion chamber and the perfusate was collected at 5-min intervals. The quantity of BDNF in each fraction was determined by a 2-site immunoassay.¹⁰⁻¹³

We initially analyzed the role of high-potassium (40 mM KCl) that was described previously to promote secretion of gliotransmitters in cultured astrocytes.^{14,15}

CONTACT Beatrice Vignoli  beatrice.vignoli@unitn.it

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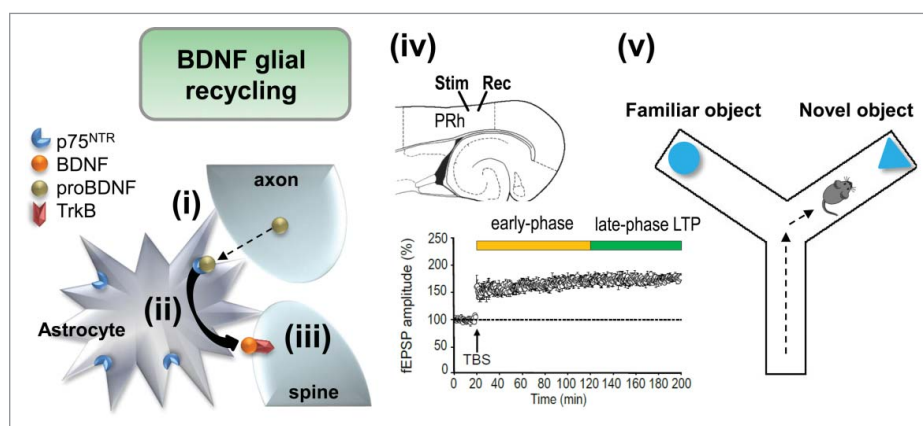


Figure 1. Long-term memory retention requires synaptic glia for proBDNF uptake and recycling. Subsequent relevant steps account for the recycling process: (i) proBDNF is secreted from neurons and internalized into peri-synaptic glia *via* p75^{NTR} following LTP-inducing electrical stimulation; (ii) endocytic vesicles containing p75^{NTR}/proBDNF reside at peri-synaptic glia before routing to the secretory pathway; (iii) recycled proBDNF is processed to mature neurotrophin inducing TrkB phosphorylation in nearby neurons; (iv) which fulfills the function to mediate the switch from, early- to late-phase LTP; (v) BDNF glial recycling in astrocytes can profoundly impact visual object recognition memory.

This stimulation is also known to regulate the secretion of BDNF from neurons through a mechanism involving depolarization and calcium increase,^{12,16-19} but whether it could also cause endocytic BDNF secretion by astrocytes is unclear. Treating cells for 5 min with KCl increased BDNF immunoreactivity (Fig. 2). The release of BDNF augmented rapidly, with maximum levels in the perfusate being reached 5 min after the beginning of KCl administration. This indicates that depolarization regulates endocytic BDNF secretion in astrocytes.

Given high-potassium is known to increase intracellular calcium levels through the activation of voltage-gated calcium channels in cultured astrocytes,²⁰ we next sought to investigate whether the exposure of cortical astrocytes

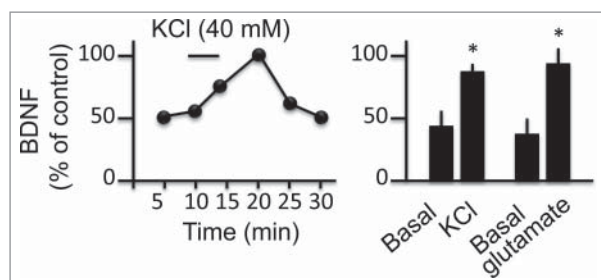


Figure 2. KCl⁻ and glutamate-mediated depolarization induce the release of endocytic BDNF in cortical astrocytes. (Left) Astrocytes previously exposed to BDNF (mix) for 10 min were perfused and stimulated with KCl (40 mM) for 5 min. ELISA quantification of BDNF secretion from perfusates showed levels of BDNF before (0 to 10 min) during (10 to 15 min) and after (15 to 30 min) KCl application. (Right) This panel shows BDNF levels measured in the 5-min fraction collected after KCl (40 mM) or glutamate (500 μ M) stimulation. Data are means \pm SEM (error bars). *, $P \leq 0.05$.

to stimuli known to raise intracellular calcium could mimic the effect of high-potassium on BDNF release. In support of this hypothesis, glutamate (500 μ M) applied for 5 min to BDNF (mix)-treated astrocytes showed to trigger endocytic BDNF secretion (Fig. 2).³ The extent of BDNF secretion elicited by glutamate was similar to that observed after treatment with KCl, and BDNF levels peaked in the same fraction. Thus, astrocytes can provide endocytic BDNF secretion by sensing transmitter-mediated depolarization. This suggests that in physiologic conditions gliotransmitters rather than voltage-dependent excitation induce astrocyte depolarization,²¹ and BDNF secretion. In line with this idea, we have previously demonstrated that while high frequency (50 Hz) electrical stimulation induces endocytic BDNF secretion in cultured neurons,¹¹ the same stimulation in cultured astrocytes was not effective.³ Our data clearly indicate that electrical activity is not sufficient to induce endocytic BDNF secretion from glial cells.³

ATP-mediated release of endocytic BDNF in cortical astrocytes

While secretion of endocytic BDNF from astrocytes uses glutamate as exogenous stimuli,³ this is not necessarily restricted to this type of transmitter; rather it is likely expected that similar secretion of endocytic BDNF can be triggered by different transmitters. This prompted us to investigate whether cultured astrocytes could eventually release endocytic BDNF upon depolarizing conditions triggered by the extracellular nucleotides ATP, a well-known signaling molecule participating in synaptic transmission.

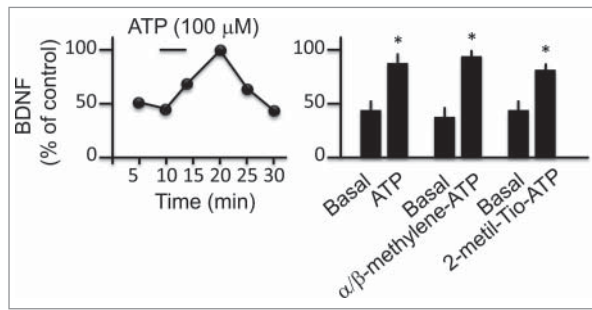


Figure 3. ATP- and ATP analogs-mediated release of endocytic BDNF in cortical astrocytes (Left) Astrocytes previously exposed to BDNF (mix) for 10 min were perfused and stimulated with ATP (100 μ M) for 5 min. ELISA quantification of BDNF secretion from perfusates showed levels of BDNF before (0 to 10 min) during (10 to 15 min) and after (15 to 30 min) ATP application. (Right) Increased BDNF secretion induced by ATP (100 μ M), α/β -methylene-ATP (50 μ M) or 2-metil-Tio-ATP (50 μ M). Data are means \pm SEM (error bars). *, $P \leq 0.05$.

To address whether ATP may serve as a ligand for glial purinergic receptors that trigger endocytic BDNF secretion, we incubated astrocyte cultures for 10 min with exogenous BDNF (mix) and subsequently stimulated with ATP (100 μ M) for 5 min (Fig. 3). This elicited an increase in endocytic BDNF secretion that was comparable to that induced by glutamate administration (Fig. 2), providing evidence that both transmitters induce endocytic BDNF secretion with similar efficacy. To obtain further insight into the mechanism of ATP-induced BDNF secretion

from astrocytes, we used the ATP analogs 2-metil-Tio-ATP (50 μ M) or α/β -methylene-ATP (50 μ M), specific ligands for ionotropic P2X or metabotropic P2Y purinergic receptors, respectively. Addition of ATP-analogs to BDNF (mix)-treated astrocytes produced a significant increase of BDNF in the perfusates (Fig. 3). Our results indicate that P2X and P2Y receptors enable astrocytes to respond to ATP finally promoting BDNF release through calcium elevation, a mechanism that was previously observed in microglia.²² However, what intracellular signaling are involved in the release process are hotly debated issues that will require further investigations.

The extracellular nucleotide ATP is known to co-localize with conventional neurotransmitters at cortical synapses, from which it can be released upon presynaptic depolarizing stimuli or can be secreted from post-synaptic sites.²³ Astrocytes get in direct contact with the released neurotransmitters,²⁴ suggesting that secretion of endocytic BDNF from glial cells is potentially triggered by ATP. In the tripartite hypothesis of synaptic transmission, where astrocytes form a third effective component of the synapse,²⁵ glutamate and ATP trafficking from neurons to astrocytes can play a complex regulatory signaling for maintaining sufficient levels of BDNF according to synaptic requirements (Fig. 4). As glial vesicular release of BDNF is regulated by neuronal activity,²¹ astrocytes may then couple neuronal network activity to the local synaptic need of the neurotrophin. Such “on

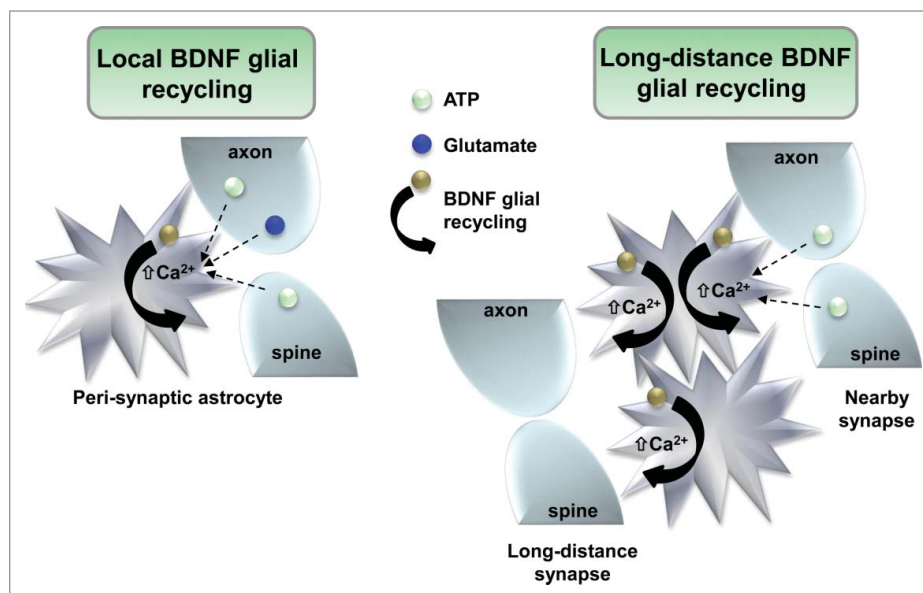


Figure 4. Local and long-distance BDNF recycling from astrocytes (left) Glioactive glutamate and/or ATP promote local BDNF recycling at glial endfeet by increasing intracellular calcium (Ca^{2+}). While glutamate can be released from pre-synaptic-terminals, ATP release could conceivably occur from both pre- and post-synaptic-terminals. (right) Glioactive ATP promotes local elevation of intracellular calcium that afterward spread to different endfeet of the same astrocyte and/or adjacent astrocytes. This raises the possibility that ATP enable glial cells to function as cellular network competent for BDNF glial recycling at nearby or long-distance synapses.

demand” supply of BDNF may serve to recruit active synapses at behaviorally relevant circuits; as those contributing to the recognition of learned objects in perirhinal cortex.¹ Overall, our results indicate that neurons may regulate glial BDNF recycling *via* the release of distinct transmitters, suggesting that neuron-astrocyte interaction can play a much more intricate and functional role in information processing coupled to cognitive functions than previously expected.

Glial cells are also skilled of inducing a local elevation of intracellular calcium. Surprisingly, whereas the calcium signals in glial cells is assumed to be dependent on IP₃-mediated release from internal stores, very recent data demonstrated that cortical astrocyte signals depend on calcium influx through ATP-gated P₂×1R ion channels.²⁶ This calcium elevation occurs in astrocyte fine processes, which lack IP₃ receptors and in which sensory stimulation evokes rapid increases in astrocytic calcium that precede increases in blood flow.²⁶ These new data hint at the possibility that the calcium-dependent signaling pathways in different endfeet of the same astrocyte, which enwrap multiple distinct synapses, may be recruited differently according to network activity. Moreover, local calcium afterward spread (calcium waves) to adjacent astrocytes. Calcium waves are thought to propagate as a result of diffusible ATP that is required for distant calcium signaling.²⁷ This raises the possibility that ATP enable glial cells to function as cellular network competent for information processing and storage, features that are commonly assigned to neurons.²⁸ At the network level, the electrical stimulation of afferents is likely to activate several types of neurons and glial cells and, concomitantly, the release of ATP. Our results that ATP mediates endocytic BDNF secretion raise the possibility that glial network, with precise temporal and spatial regulation of BDNF release, actively participate in information processing and memory storage together with neuronal network (Fig. 4). Reciprocal interactions between glial and neuronal networks may thus strengthen the ability to organize memory, and considerably expand storage capabilities. Whether BDNF glial recycling could act, as signaling mechanism involved in neuronal to glial network communication in the cortex and *vice-versa* is an attractive hypothesis that remains to be elucidated.

Material and methods

Release experiments were performed as described previously;¹⁰ stimulations were obtained by high potassium (40 mM), glutamate (500 μM), ATP (100 μM) and the ATP analogs α/β -methylene-ATP (50 μM) or 2-methyl-Tio-ATP (50 μM). Treatment started 10 min after the beginning of perfusion and was maintained over a 5-min

period. Two-site ELISA was used to determine the amount of BDNF in each fraction.^{10-13,29}

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

- [1] Vignoli B, Battistini G, Melani R, Blum R, Santi S, Berardi N, Canossa M. Peri-synaptic glia recycles brain-derived neurotrophic factor for LTP stabilization and memory retention. *Neuron* 2016; 92:873-87; PMID:27746130; <http://dx.doi.org/10.1016/j.neuron.2016.09.031>
- [2] Zuccaro E, Bergami M, Vignoli B, Bony G, Pierchala BA, Santi S, Cancedda L, Canossa M. Polarized expression of p75NTR specifies axons during development and adult neurogenesis. *Cell Rep* 2014; 7:138-52; PMID:24685135; <http://dx.doi.org/10.1016/j.celrep.2014.02.039>
- [3] Bergami M, Santi S, Formaggio E, Cagnoli C, Verderio C, Blum R, Berninger B, Matteoli M, Canossa M. Uptake and recycling of pro-BDNF for transmitter-induced secretion by cortical astrocytes. *J Cell Biol* 2008; 183:213-21; PMID:18852301; <http://dx.doi.org/10.1083/jcb.200806137>
- [4] Bergami M, Rimondini R, Santi S, Blum R, Götz M, Canossa M. Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. *Proc Natl Acad Sci USA* 2008; 105:15570-5; PMID:18832146; <http://dx.doi.org/10.1073/pnas.0803702105>
- [5] Bergami M, Vignoli B, Motori E, Pifferi S, Zuccaro E, Menini A, Canossa M. TrkB signaling directs the incorporation of newly generated periglomerular cells in the adult olfactory bulb. *J Neurosci* 2013; 33:11464-78; PMID:23843518; <http://dx.doi.org/10.1523/JNEUROSCI.4812-12.2013>
- [6] Bergami M, Berninger B, Canossa M. Conditional deletion of TrkB alters adult hippocampal neurogenesis and anxiety-related behavior. *Commun Integr Biol* 2009; 2:14-6; PMID:19704856; <http://dx.doi.org/10.4161/cib.2.1.7349>
- [7] Canossa M, Rovelli G, Shooter EM. Transphosphorylation of the neurotrophin Trk receptors. *J Biol Chem* 1996; 271:5812-8; PMID:8621450; <http://dx.doi.org/10.1074/jbc.271.10.5812>
- [8] Rovelli G, Heller RA, Canossa M, Shooter EM. Chimeric tumor necrosis factor-TrkA receptors reveal that ligand-

- dependent activation of the TrkA tyrosine kinase is sufficient for differentiation and survival of PC12 cells. *Proc Natl Acad Sci USA* 1993; 90:8717-21; PMID:7690970; <http://dx.doi.org/10.1073/pnas.90.18.8717>
- [9] Agulhon C, Petravicz J, McMullen AB, Sweger EJ, Minton SK, Taves SR, Casper KB, Fiacco TA, McCarthy KD. What is the role of astrocyte calcium in neurophysiology? *Neuron* 2008; 59:932-46; PMID:18817732; <http://dx.doi.org/10.1016/j.neuron.2008.09.004>
- [10] Canossa M, Griesbeck O, Berninger B, Campana G, Kolbeck R, Thoenen H. Neurotrophin release by neurotrophins: implications for activity-dependent neuronal plasticity. *Proc Natl Acad Sci USA* 1997; 94:13279-86; PMID:9371837; <http://dx.doi.org/10.1073/pnas.94.24.13279>
- [11] Santi S, Cappello S, Riccio M, Bergami M, Aicardi G, Schenk U, Matteoli M, Canossa M. Hippocampal neurons recycle BDNF for activity-dependent secretion and LTP maintenance. *EMBO J* 2006; 25:4372-80; PMID:16957779; <http://dx.doi.org/10.1038/sj.emboj.7601303>
- [12] Canossa M, Gärtner A, Campana G, Inagaki N, Thoenen H. Regulated secretion of neurotrophins by metabotropic glutamate group I (mGluRI) and Trk receptor activation is mediated via phospholipase C signalling pathways. *EMBO J* 2001; 20:1640-50; PMID:11285228; <http://dx.doi.org/10.1093/emboj/20.7.1640>
- [13] Aicardi G, Argilli E, Cappello S, Santi S, Riccio M, Thoenen H, Canossa M. Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. *Proc Natl Acad Sci USA* 2004; 101:15788-92; PMID:15505222; <http://dx.doi.org/10.1073/pnas.0406960101>
- [14] Rutledge EM, Kimelberg HK. Release of [3H]-D-aspartate from primary astrocyte cultures in response to raised external potassium. *J Neurosci* 1996; 16:7803-11. PMID:8987808
- [15] Yaguchi T, Nishizaki T. Extracellular high K⁺ stimulates vesicular glutamate release from astrocytes by activating voltage-dependent calcium channels. *J Cell Physiol* 2010; 225:512-8; PMID:20506270; <http://dx.doi.org/10.1002/jcp.22231>
- [16] Blöchl A, Thoenen H. Characterization of nerve growth factor (NGF) release from hippocampal neurons: evidence for a constitutive and an unconventional sodium-dependent regulated pathway. *Eur J Neurosci* 1995; 7:1220-8; PMID:7582095; <http://dx.doi.org/10.1111/j.1460-9568.1995.tb01112.x>
- [17] Griesbeck O, Canossa M, Campana G, Gärtner A, Hoener MC, Nawa H, Kolbeck R, Thoenen H. Are there differences between the secretion characteristics of NGF and BDNF? Implications for the modulatory role of neurotrophins in activity-dependent neuronal plasticity. *Microsc Res Tech* 1991; 45:262-75; PMID:10383119; [http://dx.doi.org/10.1002/\(SICI\)1097-0029\(19990515/01\)45:4/5%3c262::AID-JEMT10%3e3.0.CO;2-K](http://dx.doi.org/10.1002/(SICI)1097-0029(19990515/01)45:4/5%3c262::AID-JEMT10%3e3.0.CO;2-K)
- [18] Balkowiec A, Katz DM. Cellular mechanisms regulating activity-dependent release of native brain-derived neurotrophic factor from hippocampal neurons. *J Neurosci* 2002; 22:10399-407; PMID:12451139
- [19] Wang X, Butowt R, Vasko MR, von Bartheld CS. Mechanisms of the release of anterogradely transported neurotrophin-3 from axon terminals. *J Neurosci* 2002; 22:931-45; PMID:11826122
- [20] MacVicar BA, Hochman D, Delay MJ, Weiss S. Modulation of intracellular Ca²⁺ in cultured astrocytes by influx through voltage-activated Ca²⁺ channels. *Glia* 1991; 4:448-55; PMID:1718862; <http://dx.doi.org/10.1002/glia.440040504>
- [21] Bezzi P, Volterra A. A neuron-glia signalling network in the active brain. *Curr Opin Neurobiol* 2001; 11:387-94; PMID:11399439; [http://dx.doi.org/10.1016/S0959-4388\(00\)00223-3](http://dx.doi.org/10.1016/S0959-4388(00)00223-3)
- [22] Trang T, Beggs S, Wan X, Salter MW. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. *J Neurosci* 2009; 29(11):3518-28; <http://dx.doi.org/10.1523/JNEUROSCI.5714-08.2009>
- [23] Illes P, Ribeiro JA. Neuronal P2 receptors of the central nervous system. *Curr Top Med Chem* 2004; 4:831-8; PMID:15078214; <http://dx.doi.org/10.2174/1568026043451032>
- [24] Derouiche A, Anlauf E, Aumann G, Mühlstädt B, Lavielle M. Anatomical aspects of glia-synapse interaction: the perisynaptic glial sheath consists of a specialized astrocyte compartment. *J Physiol Paris* 2002; 96:177-82; PMID:12445894; [http://dx.doi.org/10.1016/S0928-4257\(02\)00004-9](http://dx.doi.org/10.1016/S0928-4257(02)00004-9)
- [25] Araque A, Sanzgiri RP, Parpura V, Haydon PG. Astrocyte-induced modulation of synaptic transmission. *Can J Physiol Pharmacol* 1999; 77:699-706; PMID:10566947; <http://dx.doi.org/10.1139/y99-076>
- [26] Mishra A, Reynolds JP, Chen Y, Gourine AV, Rusakov DA, Attwell D. Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. *Nat Neurosci* 2016; 19:1619-1627; PMID:27775719; <http://dx.doi.org/10.1038/nn.4428>
- [27] Abbracchio MP, Ceruti S. Roles of P2 receptors in glial cells: focus on astrocytes. *Purin Signal* 2006; 2:595-604; <http://dx.doi.org/10.1007/s11302-006-9016-0>
- [28] Perea G, Araque A. Synaptic information processing by astrocytes. *J Physiol Paris* 99:92-7; PMID:16442272; <http://dx.doi.org/10.1016/j.jphysparis.2005.12.003>
- [29] Canossa M, Giordano E, Cappello S, Guarnieri C, Ferri S. Nitric oxide down-regulates brain-derived neurotrophic factor secretion in cultured hippocampal neurons. *Proc Natl Acad Sci USA* 2002; 99:3282-7; PMID:11867712; <http://dx.doi.org/10.1073/pnas.042504299>