Astrocyte-endotheliocyte axis in the regulation of the blood-brain barrier

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Abstract

The evolution of blood-brain barrier paralleled centralisation of the nervous system: emergence of neuronal masses required control over composition of the interstitial fluids. The barriers were initially created by glial cells, which employed septate junctions to restrict paracellular diffusion in the invertebrates and tight junctions in some early vertebrates. The endothelial barrier, secured by tight and adherent junctions emerged in vertebrates and is common in mammals. Astrocytes form the parenchymal part of the blood-brain barrier and commutate with endothelial cells through secretion of growth factors, morphogens and extracellular vesicles. These secreted factors control the integrity of the blood-brain barrier through regulation of expression of tight junction proteins. The astrocyte-endotheliocyte communications are particularly important in various neurological diseases associated with impairments to the blood-brain barrier. Molecular mechanisms supporting astrocyte-endotheliocyte axis in health and disease are in need of detailed characterisation.

Key words: Blood-brain barrier, astrocyte, endothelial cells, astrocytic endfeet; tight junctions
Blood-brain barrier: discovery, evolutionary perspective and anatomy

A system of several cellular barriers separates the central nervous system (CNS) from the rest of the body. These barriers regulate molecular exchange between the blood and the nervous tissue being thus a fundamental part of the CNS homeostasis. Three main barrier systems existing in the CNS of mammals include: (1) the choroid plexus blood—cerebrospinal fluid (CSF) barrier in the ventricles of the brain, formed by tight junctions between the choroid plexus cells; (2) the arachnoid blood—CSF barrier separating the subarachnoid CSF from the blood and formed by tight junctions between the cells of the arachnoid mater surrounding the brain; and (3) the blood—brain barrier (BBB) between the blood vessels and the parenchyma of the CNS, formed by tight junctions between the epithelial cells of the blood vessels [1].

The concept of the specialised barrier between CNS and the circulating blood evolved from early experiments of Paul Ehrlich who discovered that injection of soluble dyes into the blood stream labels all tissues except the brain and the spinal cord [2]. Ehrlich, however, did not introduce the idea of special barrier; moreover he explicitly rejected the notion of different permeability of capillaries in different organs [3], see also [4]. Erlich's pupil Edwin Goldmann introduced the term Physiologische Grenzmembran or tissue border membrane [5], after he injected trypan blue into the cerebrospinal fluid; this procedure stained solely the brain and the spinal cord but not other tissues. Max Lewandowsky [6] performed functional experiments by injecting strychnine and sodium ferrocyanide either into the blood or directly into the nervous tissue; in the latter case both poisons were 100 times more effective. Lewandowsky thus suggested that capillaries blocked transport of these molecules into the brain, and was credited with the invention the term Bluthirnschranke, or blood-brain border, which however can not be found in his original writings [4]. The concept of blood-brain barrier was formalised by Lina Stern, who named it barrière hémato-encéphalique and who introduced the terms "barrier selectivity" and "barrier resistance" [7-9].

The cellular barriers separating the circulation (of blood or haemolymph) from the nervous tissue appeared early in evolution (Fig.1) [10-12]; the emergence of these barriers accompanied (and was most likely prompted by) the centralisation of the nervous system and appearance of substantial neuronal masses in the form of fused ganglia. In the invertebrates the functional barrier separating haemolymph from the brain is formed exclusively by glial cells. In Drosophila, for example, the haemolymph-brain barrier is made from two closely associated types of glia: the perineural and subperineural glial cells. The perineural glia covers the whole nervous system, whereas subperineural glial cells, placed immediately beneath the perineural glia form the restrictive barrier [13]. The subperineural glial cells are few in number, are very large in their surface, and are very thin (< 1 μm) [14-16]. The glial barrier is sealed by numerous septate junctions formed between subperineural glial cells. The septate junctions are complex comb-like structures made from several tens of proteins (in Drosophila, for example, 24 such proteins have been identified [13]) that connect adjacent cells membranes separated by ~20 nm gap [17]. The septate junctions effectively prevent paracellular transport through subperineural glia layer. The subperineural glial cells are also connected by innexin-made
gap junctions that allow intercellular diffusion of molecules and sustain propagation of Ca\(^{2+}\) waves [18-20]. The subperineural glial cells express multiple transporters that define the barrier selectivity [21]. The glial barriers are particularly important for screening the CNS milieu from the excessive K\(^+\) concentration: K\(^+\) concentration in the haemolymph in Drosophila is around 25 - 30 mM, whereas in the CNS K\(^+\) concentration is kept below 5 mM. The glial barriers are functional in other higher invertebrates such as Cephalopoda (for example in cuttlefish) [10, 22], although there are some evidence for the existence of vertebrate-like barrier in Octopus vulgaris; in particular the transcriptome of this cephalopod contains several genes involved in formation of tight junctions [23].

The glial blood-brain barrier exists also in some, but not in all, early vertebrates. For example Elasmobranchii (rays, skates and sharks), have glial blood-brain barrier sealed by tight junctions. In sharks, in particular, astrocytes can fully encircle blood vessels making them endocellular capillaries [24]. The glial blood-brain barrier also operates in some higher species such as sturgeon (which belongs to the Chondrostei subclass of bony fishes). In sturgeon blood vessels are surrounded by a complex multilayer glial sheath joined through numerous gap junctions without obvious tight junctions [11]. At the same time, more archaic forms of vertebrates, such as hagfish, lampreys (Cephalaspidomorphi), bichir and chimaeras have endothelial barrier [11, 25]. Most likely endothelial barrier represents the advancement of the glial one. The endothelial barrier occurred in evolution several times; glial cells, however, retain some barrier capabilities, which they utilise in certain brain regions, in development and in pathology [11]. In Teleostei (zebrafishes) the barrier rests with endothelium and is sealed with tight junctions; the glial coverage is however limited - the processes of radial glia have been identified close to the blood vessels, without however ensheathing them [26].

The BBB in mammals includes several components (Fig. 2): (i) tunica intima represented by endothelial cells anchored to the basal lamina, which, in turn, is a part of vascular basement membrane formed from extracellular matrix proteins secreted by endothelial cells and pericytes; (ii) smooth muscle cells surrounding large vessels; (iii) pericytes acting as contractile components in capillaries, (iv) parenchymal basal membrane made from extracellular matrix secreted by astrocytes and (v) astrocytic endfeet, which provide almost complete coverage of brain vasculature and, together with parenchymal basement membrane form glia limitans vascularis [27, 28].

**Endothelial cells form vascular barrier**

The human brain, together with liver and gastro-intestinal system, is characterised by high energy demand; the brain absorbs ~ 20% of base metabolic rate, consuming ~ 20% of oxygen and glucose needed for sustaining the organism. To satisfy energy demand the brain is highly vascularised, with total length of brain vessels in the adult human brain approaching 600 km and total surface area of these vessels being in the range of 15 - 25 m\(^2\) [29]. Endothelial cells, which form the haemocompatible surface of the luminal walls of blood vessels, are simple squamous epithelial cells of mesodermal origin [30]. The total number of endothelial cells in the human body is estimated at 1 - 6 x 10\(^{13}\); with an average surface area of all vasculature intima ~ 3000 - 6000 m\(^2\) [31] hence the human
brain contains at least 5 - 10 billions of endothelial cells, making them the third most
umerous after neurones and neuroglia.

The endothelial cells are usually oriented along the axis of the vessels, average length of
endotheliocytes is 30 - 50 µm, width of 10 - 50 µm and thickness between 0.1 and 10 µm
[31]. There is quite a degree of morphological heterogeneity between endothelial cells
from different parts of the vasculature. The smallest capillaries of the brain, for example,
are lined with a single endothelial cell with < 0.5 µm distance between luminal and
parenchymal surfaces [32]. When being maintained in vitro, endothelial cells form a
monolayer with characteristic cobble-stone appearance, revealed by phase-contrast
microscopy or by immunoassaying with specific antibodies (Fig. 3). The brain
endothelial cells are clamped together with tight junctions and adherent junctions, which
define barrier resistance and prevent paracellular flux of hydrophilic molecules into the
nervous tissue. The adherent junctions are cell-to-cell adhesive complexes, being in
essence a plasma membrane associated organelles, of which zonula adherens is probably
the most abundant [33]. The adherent junctions complexes are mainly build from
cadherin adhesion molecules [34]. In physiological conditions, cortical actin bundles
control equal distribution of adherent junctions between neighbouring cells. In pathology
however, various permeability-increasing factors, such as thrombin, trigger actin
remodelling leading to the relocation of vascular-endothelial (VE)-cadherin complexes
and adherent junctions thus disrupting the latter and consequently compromising the
integrity of blood-brain barrier [35, 36]. Tight junctions clamp together plasma
membranes of neighbouring cells through electron-dense junctional plaques made from
several proteins, including occludin and claudins associated with zonula occludens (ZO -
1,2,3) proteins, which localise to the cytoplasmic part of the plasma membrane and
anchor tight junction complexes to the actin filaments [28]. In endothelial monolayers in
vitro all these proteins show clear localisation to the plasma membranes (Fig. 4). The
tight junctions restrict paracellular transport, including ions, which defines a very high
(1000 - 5000 Ω/cm²) transendothelial electrical resistance (TEER) of the brain
endothelial barrier, which is >100 times larger as compared to peripheral capillaries
where TEER varies between 2 and 20 Ω/cm² [37, 38].

The selectivity of endothelial barrier is determined by the complement of transporters; the
transcytosis in the brain endothelial cells seems to be limited [27]. Gases (primarily
oxygen and carbon dyoxide) traverse lipid membrane with ease; the same applies to some
small lipid-soluble molecules. Translocation of hydrophilic molecules is restricted by
complement of luminal and abluminal membrane transporters; the array of ecto- and
intracellular enzymes metabolise and inactivate various neuroactive and toxic agents [1].
The efflux transporters, engaged in the export of various molecules towards the blood are
mainly represented by ATP-cassette binding transporters polarised to the luminal surface
of the endothelial barrier [39]. The nutritional transporters, which move nutritive
molecules across the BBB are represented by solute carriers, which transport glucose
(GLUT1 glucose carrier), amino acids (LAT-1/SLC7A5 large neutral amino acid
transporter), ions and vitamins, whereas larger molecules utilise the receptor mediated
transport mechanisms [40].
Astroglia form parenchymal part of the blood-brain barrier

Astroglia is a class of neural cells of ectodermal, neuroepithelial origin, which are responsible for homeostasis of the CNS. The class of astroglia includes protoplasmic astrocytes, fibrous astrocytes, surface-associated astrocytes, velate astrocytes, perivascular and marginal astrocytes, radial astrocytes, Bergmann and Müller glia, pituicytes, tanyocytes, ependymocytes, choroid plexus cells, and retinal pigment epithelial cells. The brains of higher primates and humans also contain interlaminar astrocytes, astrocytes with varicose projections and polarised astrocytes [41-44]. Although highly heterogeneous in their morphotypes astroglial cells share several core features; in particular they have high K\(^+\) membrane permeability that sets highly hyperpolarised resting membrane potential and possess extended complement of membrane transporters contributing to CNS homeostatic control [43, 45]. Astroglial cells are electrically non-excitatory; nonetheless they express "intracellular" excitability mediated by spatio-temporal fluctuations of ions and second messengers [46-48]. Protoplasmic astrocytes establish intimate contacts with neuronal synapses forming an astroglial cradle, which controls various aspects of synaptic function from synaptogenesis and synaptic maturation to synaptic isolations, maintenance and extinction [49].

Protoplasmic astrocytes in the grey matter and fibrous astrocytes send processes to the vasculature; these processes terminate with endfeet, which cover up to 98% of the entire surface of blood vessels [50, 51]. The endfeet possess rough endoplasmic reticulum which is responsible for on-site synthesis of relevant proteins [52]; endfeet are capable of secreting vasodilatating and vasoconstricting agents being thus involved in the regulation of functional hyperaemia [53]; vascular tone may also be controlled by K\(^+\) ions released through Ca\(^{2+}\)-sensitive K\(^+\) channels densely expressed in the endfeet membrane [54]. Finally, endfeet are packed with aquaporin 4 (AQP4) water channels [55], which are critical for operation of the brain-wide glymphatic system [56]. Perivascular processes of astrocytes differ depending on the vessel caliber - the processes covering arteries and veins are as a rule thicker and larger compared to the processes contacting small capillaries [57, 58].

In physiological conditions perivascular astrocytic endfeet are not tightly sealed; although they are coupled with gap junctions, which facilitate intercellular signalling through parenchymal portion of the BBB [59], and are somehow linked to BBB permeability [60]. In pathological conditions associated with the BBB breakdown, however, endfeet of reactive astrocytes mount a parenchymal line of defence by starting to express tight junctions which seal the endfeet barrier [61, 62]. In particular astrocytes begin to synthesise canonical tight junction proteins claudin 1, claudin 4, and junctional adhesion molecule-A [61]. It has to be noted that specific astroglial cells, the tanyocytes of the hypothalamus, create the blood brain barrier by tight junctions clamping their somata [63].

Role of astrocytes in regulation of BBB
All cellular elements of the BBB are integrated into the neurogliovascular unit, which binds together endothelial cells, pericytes, vascular smooth muscle cells, astrocytes, microglia and neurones [28, 43]. At the capillary level, neurogliovascular unit is composed of endothelial cells sharing a common basement membrane with pericytes and astrocytic endfeet. At the level of arterioles and venulae endothelial cells are surrounded by vascular basement membrane, several layers of smooth muscle cells and parenchymal basement membrane associated with astrocyte endfeet [28]. The parenchymal basement membrane represented by extracellular matrix produced by astrocytes is morphologically indistinguishable from the endothelial basement membrane, although it has distinct molecular composition. Endothelial cells secrete laminin-411 and laminin-511, whereas astrocytes release laminin-111 and laminin -211 [64].

Several studies demonstrated the importance of astrocytic laminins for the proper functioning of the BBB. Ablation of astrocytic laminin by tissue-specific Cre-mediated recombination affected smooth muscle cells and led to brain haemorrhages in adult mice [65]. Astrocytic laminins regulate pericyte differentiation and influence the expression and polarisation of AQP4 to the endfeet as well as expression of tight junction proteins in endotheliocytes [66]. The perivascular space (also known as a Virchow–Robin space), surrounding larger caliber vessels, is used by glymphatic clearance system to eliminate waste products from brain parenchyma [67, 68]. In particular, astrocytic AQP4-dependent glymphatic pathway is important for the clearance of soluble β-amyloid from the interstitium. Animals lacking Aqp4 gene in astrocytes demonstrated 65 % reduction in β-amyloid clearance [56]. Operation of glymphatic system is brain-state dependent: sleep is associated with a 60% increase in the interstitial space resulting in accelerated clearance of waste protein including β-amyloid [69]. Locus coeruleus (LC) is the main noradrenergic nucleus of the CNS innervating brain precapillary and capillary vessels. At the capillary level noradrenergic terminals of LC directly contact astrocytic endfeet and pericytes and regulate neurovascular coupling [70]. In this regard it is worth noting that accumulation of hyperphosphorylated Tau proteins within LC precedes by years occurrence of neurofibrillary tangles and amyloid deposits in other brain regions [71]. Therefore it has been proposed that dysregulated LC innervation may trigger BBB dysfunction during the early stages of Alzheimer’s disease (AD) [70].

Astrocytes represent the major source of apolipoprotein E (ApoE) in the brain. Experiments with different APOE transgenic mice revealed that expression of human APOE4 isoform, which is a major genetic risk factor for AD, disrupts integrity of blood-brain barrier by activating cyclophilin A (CypA) – NF-κB - matrix metalloproteinase 9 (MMP9) pathway in pericytes [72]. A recent study demonstrated that β-amyloid and tau pathways operate independently of the CypA - MMP9 BBB breakdown pathway during the early stages of cognitive impairment in APOE4 carriers [73]. This finding further supports the role of vascular contribution and two-hit hypothesis stating that neurovascular dysfunction caused by β-amyloid- independent (hit 1) genetic risk factors, vascular (hypertensia, diabetes) and environmental factors and β-amyloid- dependent (hit 2) mechanisms interact and converge on blood vessels leading to the cognitive impairment and neurodegeneration [74].
Astrocytes contribute to the regulation of neurovascular and neurometabolic coupling that occurs at the neurogliovascular unit [28]. Neurovascular coupling ensures a rapid on-demand increase of cerebral blood flow to the activated regions of brain for sufficient delivery of oxygen and glucose and simultaneous removal of metabolites such as lactic acid and carbon dioxide [74]; the phenomenon known as functional hyperaemia [75, 76]. At the arterial and arteriolar levels smooth muscle constriction is mainly regulated by the nitric oxide (NO) released from neurones, whereas at the capillary level blood flow is, in part, regulated by Ca\(^2+\)-dependent astrocytic signalling to pericytes [74]. One of the pathways is mediated by ATP released from active neurones; ATP in turn activates astrocytic P2X receptors which trigger Ca\(^{2+}\) signals that induce phospholipase D2 (PLD2) and diacylglycerol lipase (DAG lipase)-mediated production of arachidonic acid (AA) and generation of prostaglandin E\(_2\) (PGE\(_2\)) by cyclooxygenase (COX1). The PGE\(_2\) secreted from astrocytic endfeet induces relaxation of pericytes by binding to the EP4 receptors [74, 77]. Astrocytes can also function as intracranial baroreceptors by detecting decrease in the brain perfusion and activating CNS autonomic sympathetic control circuits to increase systemic arterial blood pressure [78]. Acute decrease of the brain perfusion triggers astrocytic Ca\(^{2+}\) signals; this mechanosensory response may be associated with Ca\(^{2+}\)-dependent release of vasoactive signalling molecules [78]. Mechanisms regulating BBB permeability to nutrients and toxins are tightly coordinated in a bidirectional manner with neurovascular and neurometabolic coupling. The breakdown and leakage of BBB lead to neurovascular and neurometabolic uncoupling and vice versa, disturbances of blood flow (caused for example by vasoconstriction induced by a local ischemia) may increase permeability of BBB to the toxic blood components [79, 80].

Astrocytes secrete numerous paracrine factors supporting and affecting barrier properties of the BBB [81, 82]. At the neurogliovascular unit these factors act mainly through interactions with endothelial cells by regulating expression and distribution of tight junction and adherent junction proteins [83]. In addition astrocytes interact with pericytes through CypA - MMP9 signalling pathway [72, 73]. Among identified astrocytic paracrine factors are morphogens Sonic hedgehog (Shh) and Wnt, fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), angiopoetins, glial cell line-derived neurotrophic factor (GDNF), ApoE and others [28, 83].

In addition, astrocytes secrete different types of extracellular vesicles (EVs) that can transport various miRNAs, proteins, lipids and metabolites [84]. Astrocytic EVs carry FGF2, VEGF, apolipoprotein-D, synapsin1, Hsp70 chaperone, glutamate transporters EAAT-1 and EAAT-2 [85-87]. However, the role of astroglia-secreted EVs in the regulation of the BBB integrity remains elusive. We have recently compared effects of EVs derived from immortalised astrocytes prepared from hippocampi of triple transgenic AD model mouse (3xTG-AD) and wild-type controls [88] and found (Fig. 5) that treatment with EVs from healthy astrocytes increased trans-endothelial electrical resistance and up-regulated expression of tight junction proteins in monolayers of human brain endothelial cells (hCMEC/D3), whereas EVs from 3Tg-iAstro were ineffective [89]. More studies are needed to address specific effects of human astrocyte EVs in humanised disease-specific in vitro models of BBB. The EVs also act as transporters for
lipid-modified hydrophobic morphogens Wnt and Shh through hydrophilic environments [90, 91]. We therefore suggest that the same mechanisms can be employed by astrocytes secreting EVs for transport of morphogens to the adjacent cells at the neuro-gliovascular unit [85]. It needs to be considered, however, that the same signalling molecules may induce different functional responses in target cells when presented in soluble form, or as cargo components of EVs.

Many paracrine factors secreted by astrocytes and previously reported as modulators of BBB in vitro are expressed at a very low or undetectable levels in the astrocytes in the healthy brain. Various lesions to the nervous tissue trigger powerful defensive response of astrocytes known as reactive astrogliosis, which results in emergence of many context- and disease-specific reactive phenotypes [92]. In particular, reactive astrogliosis remodels astrocytic secretome so that numerous signalling molecules aimed at orchestrating brain defence are released [93]. For example, Shh secreted from reactive astrocytes promotes BBB integrity following injury through interactions with Hedgehog (Hh) receptors in endotheliocytes [94]. In the healthy brain, however, the Shh is mostly expressed in the neurones of the cerebral cortex and it has been rarely detected in astrocytes [95]. Similarly, expression of VEGF-A was undetectable in the astrocytes of control animals whereas interleukin-1β (IL-1β) induced expression of VEGF-A in the reactive astrocytes leading to the eNOS-dependent down-regulation of tight junction proteins in endotheliocytes and disruption of BBB [96]. Thus, so far little is known about the paracrine factors secreted by astrocytes in the unperturbed adult brain. Likewise, the role of astrocytes in maintenance of the BBB in the adult and aging brain remains largely unknown. Using astrocyte-specific and sensitive loss- and gain-of-function genetic approaches may help to address these issues.

In a mouse model of tamoxifen-inducible astrocyte ablation leakage of fluorescently labelled cadaverin and blood plasma fibrinogen into the brain parenchyma has been detected, indicating that astrocytes are necessary for blood-brain barrier maintenance in the adult mouse brain [97]. This conclusion however was not universally supported. In particular, in vivo acute laser ablation of astrocytic endfeet did not result in the immediate loss of the barrier integrity as judged by the absence of extravasations of plasma dyes [98]. When genetic strategy was employed for selective in vivo paralysis and depletion of GFAP-positive astrocytes, it appeared that such depletion led to the rapid neuronal loss and severe motor deficits caused by oxidative stress resulting from the reduced oxygen species scavenging capability of dysfunctional astrocytes [99]. Neuronal loss after paralysis of GFAP-positive astrocytes does not correlate with CNS vascular leakage as evidenced by lack of perivascular deposits of plasma-derived proteins, spontaneous microbleeds and leukocyte extravasation [99]. Conditional knock-out of astrocytic Wnt release led to the brain oedema and increased vascular tracer leakage accompanied with increased frequency of endothelial vesicles and caveolin-1 expression in endothelial cells [100]. In addition, disturbed Wnt secretion caused swelling of astrocytic endfeet and altered coverage of brain capillaries showing cell-autonomous role of astrocytic Wnts [100].
Similar approach was used to assess the importance of FGF signalling in the regulation of astrocyte activation after injury [101]. Deletion of FGF receptors in adult cortical astrocytes led to the astrogliotic response in the uninjured brain [101], at the same time no leakage into the cortical parenchyma was detected in the loss-of-function mutants [101]. These data demonstrate that FGF signalling suppresses astrocyte reactivity in both normal and injured brain. Different CNS pathologies have been associated with increased astrogial expression of bFGF [102-104], but how local fluctuations of bFGF affect other components of the neurogliovascular unit and blood-brain barrier integrity in vivo remains unclear. By employing a defined protocol for producing endotheliocytes from human inducible pluripotent stem cells [105] we found that autocrine secretion of bFGF is necessary for the establishment of tight endothelial barrier, whereas addition of exogenous bFGF in concentrations higher than 4 ng/ml inhibited transendothelial electric resistance of endotheliocyte monolayer in a concentration-dependent manner [37]. These findings demonstrate a dual role for bFGF in the regulation of BCEC barrier function. Future studies employing conditional knock outs of astrocytic bFGF release and (or) deletion of FGF receptors in endotheliocytes together with simultaneous in vivo monitoring of blood-brain barrier permeability could definitely clarify these issues. We believe that similar experimental approach can be used for understanding how other astrocytic paracrine factors affect blood-brain barrier in the normal and injured brain.

Conclusions

Astrocytes are indispensable part of blood-brain barrier. The endfeet of astrocytes provide an extensive coverage of brain vessels and regulate expression of endothelial tight junctions, which seal the barrier and restrict transcellular diffusion. Experimental ablation of astrocytes in the adult brain results in the damage to the endothelial barrier, highlighting the importance of astrocytic support. Astrocytes are potentially capable of creating a barrier by themselves: tight junctions between somata of tanyocytes restrict diffusion in hypothalamus lacking endothelial barrier, whereas inflammatory lesion to the endothelial barrier forces an appearance of tight junctions between astrocytic endfeet thus creating an emergency astrocytic blood-brain barrier. Specific manipulation with astrocytes therefore may represent a potential strategy for controlling the blood-brain barrier in various contexts.

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References


Figure legends

Figure 1. Evolution of the blood brain barrier from insects to mammals.

In Drosophila, the glial barrier covers the whole brain thus separating it from the haemolymph. The glial barrier is composed from two layers: the outer perineural glia and the inner subperineural glia. The latter are represented by very thin cells with large surface area with the barrier secured by septate junctions connecting glial cells. In some fishes (Elasmobrachi - sharks, skates and rays) the blood brain barrier is made by tight junctions connecting astrocytic endfeet; in sturgeons (Chondrostei) the barrier is made by several layers of closely packed glial membranes. Zebra fish (Teleostei) possesses endothelial barrier without perivascular glial coverage. In Mammalia the endothelial barrier is supported by an extensive vessel coverage with astrocytic endfeet.

Figure 2. Components of the blood-brain barrier and neurogliovascular unit (see text for explanation).

Figure 3. Representative confocal images of brain capillary endothelial cells differentiated from human inducible pluripotent stem cells and stained with antibodies against endothelial markers CD34, VE-cadherin and von Willebrand factor. Endothelial cells were differentiated from human inducible pluripotent stem cells according to the protocol described in [37, 105]. Three days before staining with antibodies against endothelial markers cells were seeded onto polyester membrane Transwell inserts coated with a mixture of 400 μg/ml collagen IV and 100 μg/ml fibronectin.

Figure 4. The upper left panel shows graphical representation of structure and molecular composition of tight junctions. Three other panels show confocal images of human brain capillary endothelial cells cultures (derived from inducible pluripotent stem cells) stained with antibodies against tight junction proteins ZO-1, occludin and claudin-5.

Figure 5. Extracellular vesicles secreted by astrocytes derived from healthy animals increase the resistance of endothelial monolayers; conversely extracellular vesicles produced by astrocytes isolated from triple transgenic AD mouse model have no effect.

A: Schematic representation of the experimental set-up. B: TEER measured after 4 days incubation with extracellular vesicles. Data represent TEER as a percentage (± SEM) relative to untreated endothelial monolayers. Statistically significant difference was determined by one way ANOVA Tukey’s test, #p < 0.0001 (n = 8 - 9).