Astrocyte–endothelial interactions at the blood–brain barrier

N. Joan Abbott, Lars Rönnbäck‡ and Elisabeth Hansson‡*

Abstract | The blood–brain barrier, which is formed by the endothelial cells that line cerebral microvessels, has an important role in maintaining a precisely regulated microenvironment for reliable neuronal signalling. At present, there is great interest in the association of brain microvessels, astrocytes and neurons to form functional 'neurovascular units', and recent studies have highlighted the importance of brain endothelial cells in this modular organization. Here, we explore specific interactions between the brain endothelium, astrocytes and neurons that may regulate blood–brain barrier function. An understanding of how these interactions are disturbed in pathological conditions could lead to the development of new protective and restorative therapies.

Neurovascular unit
A functional unit composed of groups of neurons and their associated astrocytes, interacting with smooth muscle cells and endothelial cells on the microvessels (arterioles) responsible for their blood supply, and capable of regulating the local blood flow.

Gliovascular unit
A proposed functional unit composed of single astrocytic glial cells and the neurons they surround, interacting with local segments of blood vessels, and capable of regulating blood flow at the arteriolar level and BBB functions at the capillary level.

**Wolfson Centre for Age-Related Diseases, King's College London, UK.*
‡Institute of Clinical Neuroscience, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden.
Correspondence to N.J.A.
e-mail: joan.abbott@kcl.ac.uk
doi:10.1038/nrn1824

Neuroscience has traditionally focused on the neurons of the central and peripheral nervous systems, and, increasingly, on their interactions with the glial cells that support their function. It is now becoming clear that neurons, glia and microvessels are organized into well-structured neurovascular units, which are involved in the regulation of cerebral blood flow[1]. Within this organization, further modular structure can be detected; in particular, the proposed gliovascular units, in which individual astrocytic glia support the function of particular neuronal populations and territories, and communicate with associated segments of the microvasculature[2,3]. Several recent studies have highlighted the importance of the brain endothelial cells that form the blood–brain barrier (BBB) in this modular organization, and the physiology and pharmacology of the signalling between glia and endothelium that is involved in regulating the BBB. Here, we describe the properties of the brain endothelium that contribute to its barrier function, and how cell–cell interactions lead to induction of the specialized features of the BBB and associated cell types. We review work showing that the BBB is a dynamic system, and discuss the ways in which BBB permeability and transport can be modulated. We then consider the important role of astrocytes and the BBB in brain ion and volume regulation. Finally, we discuss some of the pathologies that involve BBB dysfunction, and the development of protective strategies for the brain endothelium that may reduce secondary neural damage in both acute and chronic neurological conditions.

Barriers of the CNS

The cerebral ventricles and subarachnoid space contain cerebrospinal fluid (CSF), which is secreted by choroid plexuses in the lateral, third and fourth ventricles[4]. Three barrier layers limit and regulate molecular exchange at the interfaces between the blood and the neural tissue or its fluid spaces (FIG. 1): the BBB formed by the cerebrovascular endothelial cells between blood and brain interstitial fluid (ISF), the choroid plexus epithelium between blood and ventricular CSF, and the arachnoid epithelium between blood and subarachnoid CSF[5]. Individual neurons are rarely more than 8–20 μm from a brain capillary[6], although they may be millimetres or centimetres from a CSF compartment. Hence, of the various CNS barriers, the BBB exerts the greatest control over the immediate microenvironment of brain cells.

The blood–brain barrier

The BBB is a selective barrier formed by the endothelial cells that line cerebral microvessels[7–10] (FIG. 2). It acts as a 'physical barrier' because complex tight junctions between adjacent endothelial cells force most molecular traffic to take a transcellular route across the BBB, rather than moving paracellularly through the junctions, as in most endothelia[11,12] (FIG. 3). Small gaseous molecules such as O_2 and CO_2 can diffuse freely through the lipid membranes, and this is also a route of entry for small lipophilic agents, including drugs such as barbiturates and ethanol. The presence of specific transport systems on the luminal and abluminal membranes regulates the transcellular traffic of small hydrophilic molecules, which provides

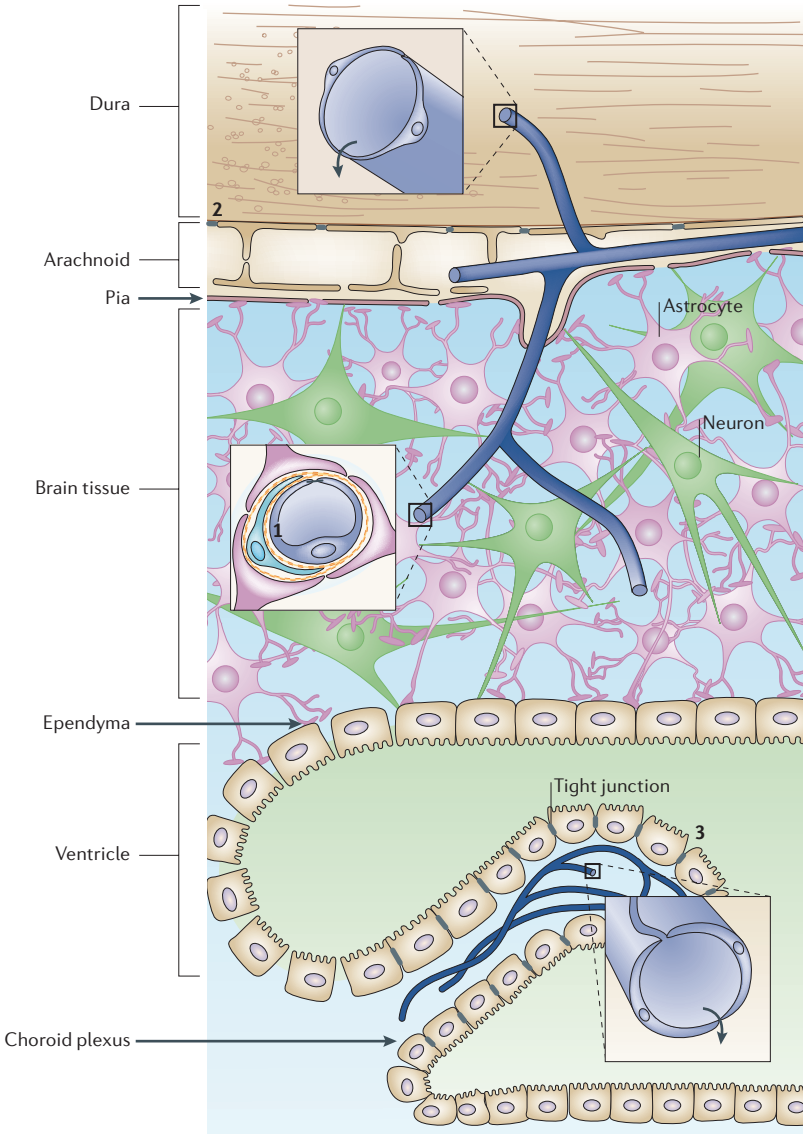

Figure 1 | **Location of barrier sites in the CNS.** Barriers are present at three main sites: the brain endothelium forming the blood–brain barrier (BBB) (**1**), the arachnoid epithelium (**2**) forming the middle layer of the meninges, and the choroid plexus epithelium (**3**), which secretes cerebrospinal fluid (CSF). At each site, the physical barrier is caused by tight junctions that reduce the permeability of the paracellular (intercellular cleft) pathway. In circumventricular organs (CVOs, not shown), which contain neurons specialized for neurosecretion and/or chemosensitivity, the endothelium is leaky. This allows tissue–blood exchange, but as these sites are separated from the rest of the brain by an external glial barrier, and from CSF by a barrier at the ependyma, CVOs do not form a leak across the BBB. Modified, with permission, from REF. 163 © (1990) Kluwer Academic.

Choroid plexus
A site of production of CSF in the adult brain. It is formed by the invagination of ependymal cells into the ventricles, which become richly vascularized.

Interstitial fluid
(ISF). The extracellular fluid filling the 'interstices' of the tissue, and bathing the cells.

a selective 'transport barrier', permitting or facilitating the entry of required nutrients, and excluding or effluxing potentially harmful compounds[10]. Finally, a combination of intracellular and extracellular enzymes provides a 'metabolic barrier': ecto-enzymes such as peptidases and nucleotidases are capable of metabolizing peptides and ATP, respectively, whereas intracellular enzymes such as monoamine oxidase and cytochrome P450 (1A and 2B) can inactivate many neuroactive and toxic compounds[13]. Large hydrophilic molecules such as peptides and proteins are generally excluded, unless they can be transferred by specific receptor-mediated transcytosis, or by the less specific adsorptive-mediated transcytosis[14]. However, the brain endothelium has a much lower degree of endocytosis/transcytosis activity than does peripheral endothelium, which contributes to the transport-barrier property of the BBB. Hence, the term 'blood–brain barrier' covers a range of passive and active features of the brain endothelium. As the tight junctions severely restrict entry of hydrophilic drugs, and there is limited penetration of larger molecules such as peptides, strategies for drug delivery to the CNS need to take these features into account.

Most studies of the BBB have concentrated on the brain capillary endothelium, the largest surface area for blood–brain exchange. Similar properties are found in the endothelium of brain arterioles and venules, although these segments of the microvasculature may be more leaky[15] and subject to greater modulation (see below).

Functions of the BBB. The BBB has several roles[8,10,16]. It supplies the brain with essential nutrients and mediates efflux of many waste products. It restricts ionic and fluid movements between the blood and the brain, allowing specific ion transporters and channels to regulate ionic traffic, to produce a brain ISF that provides an optimal medium for neuronal function[5]. ISF is similar in composition to blood plasma, but has a much lower protein content, and lower K^+ and Ca^{2+} concentrations but higher levels of Mg^{2+}. More importantly, the BBB protects the brain from fluctuations in ionic composition that can occur after a meal or exercise, which would disturb synaptic and axonal signalling[17]. The barrier helps to keep separate the pools of neurotransmitters and neuroactive agents that act centrally (in the CNS) and peripherally (in the peripheral tissues and blood), so that similar agents can be used in the two systems without 'crosstalk'. Because of its large surface area (~20 m^2 per 1.3 kg brain) and the short diffusion distance between neurons and capillaries, the endothelium has the predominant role in regulating the brain microenvironment. The choroid plexus epithelium (the blood–CSF barrier, responsible for CSF production, FIG. 1) also contributes to this process[18], as well as having other roles (for example, in growth factor secretion)[19]. Finally, continual turnover and drainage of CSF and ISF by bulk flow helps to clear larger molecules and brain metabolites, further aiding homeostasis of the brain microenvironment[5].

The BBB phenotype. A great deal is now known about specific features of the brain endothelium that contribute to its barrier properties (whether physical or chemical) and distinguish brain endothelium from the endothelium of peripheral tissues.

The tight junctions are more complex in the brain endothelium, seen in freeze–fracture images as a network of strands formed by intramembranous particles, and occlude the intercellular cleft more effectively[11,20]. These junctions significantly restrict even the movement of small ions such as Na^+ and Cl^-, so that the transendothelial electrical resistance (TEER), which is typically 2–20 ohm.cm^2 in peripheral capillaries, can be >1,000 ohm.cm^2 in brain endothelium[21].

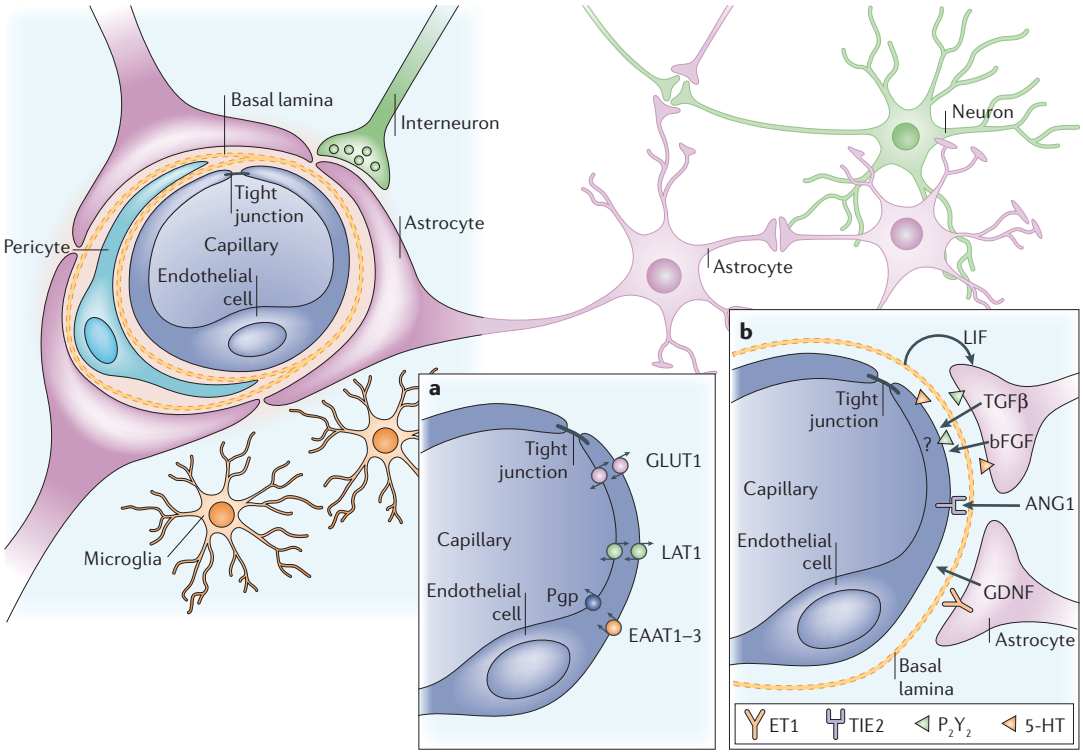

Figure 2 | **Cellular constituents of the blood–brain barrier.** The barrier is formed by capillary endothelial cells, surrounded by basal lamina and astrocytic perivascular endfeet. Astrocytes provide the cellular link to the neurons. The figure also shows pericytes and microglial cells. **a** | Brain endothelial cell features observed in cell culture. The cells express a number of transporters and receptors, some of which are shown. EAAT1–3, excitatory amino acid transporters 1–3; GLUT1, glucose transporter 1; LAT1, L-system for large neutral amino acids; Pgp, P-glycoprotein. **b** | Examples of bidirectional astroglial–endothelial induction necessary to establish and maintain the BBB. Some endothelial cell characteristics (receptors and transporters) are shown. 5-HT, 5-hydroxytryptamine (serotonin); ANG1, angiopoetin 1; bFGF, basic fibroblast growth factor; ET1, endothelin 1; GDNF, glial cell line-derived neurotrophic factor; LIF, leukaemia inhibitory factor; P_2Y_2, purinergic receptor; TGFβ, transforming growth factor-β; TIE2, endothelium-specific receptor tyrosine kinase 2. Data obtained from astroglial–endothelial co-cultures and the use of conditioned medium[8,10,24–27,33,45,50,51].

Tight junction
A belt-like region of adhesion between adjacent cells. Tight junctions regulate paracellular flux, and contribute to the maintenance of cell polarity by stopping molecules from diffusing within the plane of the membrane.

Abluminal membrane
The endothelial cell membrane that faces away from the vessel lumen, towards the brain.

Meninges
The complex arrangement of three protective membranes surrounding the brain, with a thick outer connective tissue layer (dura) overlying the barrier layer (arachnoid), and finally the thin layer covering the glia limitans (pia). The sub-arachnoid layer has a sponge-like structure filled with CSF.

Circumventricular organs
(CVOs). Brain regions that have a rich vascular plexus with a specialized arrangement of blood vessels. The junctions between the capillary endothelial cells are not tight in the blood vessels of these regions, which allows the diffusion of large molecules. These organs include the organum vasculosum of the lamina terminalis, the subfornical organ, the median eminence and the area postrema.

Receptor-mediated transcytosis
The mechanism for vesicle-mediated transfer of substances across the cell, the first step of which requires specific binding of the ligand to a membrane receptor, followed by internalization (endocytosis).

Adsorptive-mediated transcytosis
The mechanism for vesicle-mediated transfer of substances across the cell, the first step of which involves nonspecific binding of the ligand to membrane surface charges, followed by internalization (endocytosis).

Our understanding of the molecular structure of tight junctions derives from studies of both epithelia and endothelia (FIG. 4). Among the molecules identified as making important contributions to tight junction structure are the transmembrane proteins occludin and the claudins. Occludin is a 60–65 kDa protein with a carboxy (C)-terminal domain that is capable of linking with zonula occludens protein 1 (ZO-1; see below). The main function of occludin appears to be in tight junction regulation[12,22]. In the BBB, expression of the proteins claudin 3 (originally misidentified as claudin 1, now also referred to as 1/3), claudin 5 and possibly claudin 12 appears to contribute to the high TEER[11,20]. Junctional adhesion molecules JAM-A, JAM-B and JAM-C are present in brain endothelial cells, and are involved in the formation and maintenance of the tight junctions. The transmembrane proteins are connected on the cytoplasmic side to a complex array of peripheral membrane proteins that form large protein complexes, the cytoplasmic plaques. Within the plaques are adaptor proteins with many protein–protein interaction domains, including ZO-1, ZO-2 and ZO-3; the Ca^{2+}-dependent serine protein kinase (CASK); MAGI-1, MAGI-2 and MAGI-3 (membrane-associated guanylate kinase with inverted orientation of protein–protein interaction domains); the partitioning defective proteins PAR3 and PAR6; and MUPP1 (multi-PDZ-protein 1). These help to organize the second class of plaque proteins, the regulatory and signalling molecules (including the small GTPases) and their regulators, such as the regulator of G-protein signalling 5 (RGS5), and the transcription regulator the ZO-1-associated nucleic acid-binding protein (ZONAB). A newly identified protein, junction-associated coiled-coil protein (JACOP), may anchor the junctional complex to the actin cytoskeleton. Cell–cell interaction in the junctional zone is stabilized by adherens junctions.

The tight junction has a valuable function not only in restricting paracellular permeability (gate function), but also in segregating the apical and basal domains of the cell membrane (fence function) so that the endothelium can take on the polarized (apical–basal) properties that are more commonly found in epithelia, such as those of the gastrointestinal tract and kidney[20]. The PAR3–atypical protein kinase C (aPKC)–PAR6 complex appears to be involved in regulating tight junction formation and in establishing cell polarity.

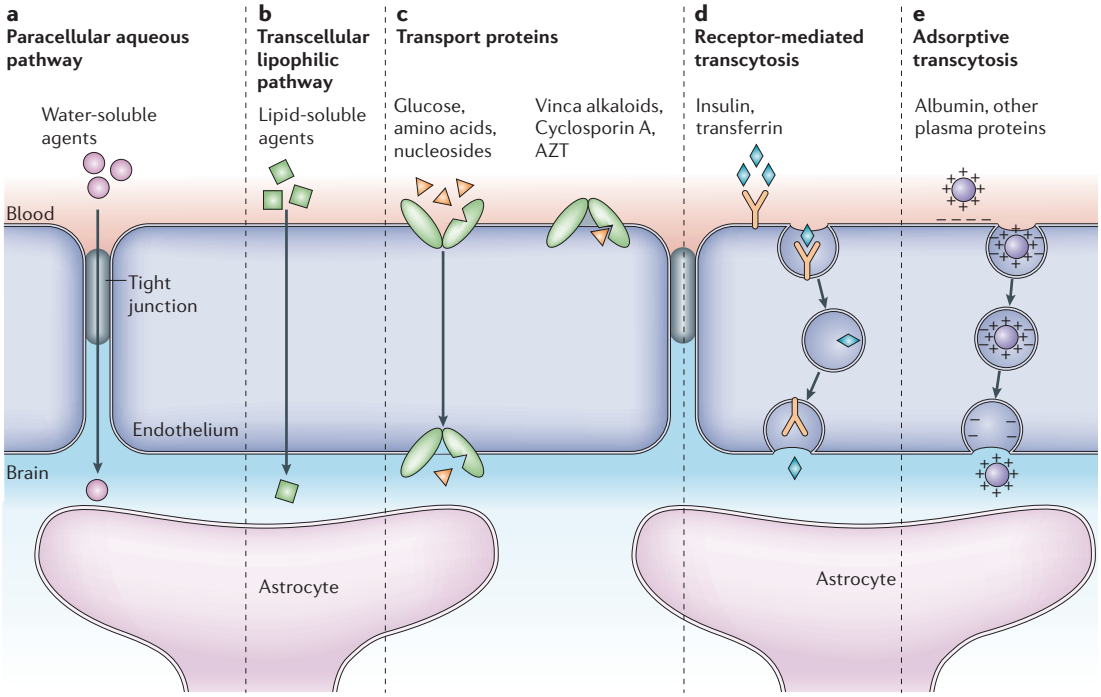

Figure 3 | **Pathways across the blood–brain barrier.** A schematic diagram of the endothelial cells that form the blood–brain barrier (BBB) and their associations with the perivascular endfeet of astrocytes. The main routes for molecular traffic across the BBB are shown. **a** | Normally, the tight junctions severely restrict penetration of water-soluble compounds, including polar drugs. **b** | However, the large surface area of the lipid membranes of the endothelium offers an effective diffusive route for lipid-soluble agents. **c** | The endothelium contains transport proteins (carriers) for glucose, amino acids, purine bases, nucleosides, choline and other substances. Some transporters are energy-dependent (for example, P-glycoprotein) and act as efflux transporters. AZT, azidothymidine. **d** | Certain proteins, such as insulin and transferrin, are taken up by specific receptor-mediated endocytosis and transcytosis. **e** | Native plasma proteins such as albumin are poorly transported, but cationization can increase their uptake by adsorptive-mediated endocytosis and transcytosis. Drug delivery across the brain endothelium depends on making use of pathways **b**–**e**; most CNS drugs enter via route **b**. Modified, with permission, from REF. 8 © (1996) Elsevier Science.

Adherens junction
A cell–cell junction also known as zonula adherens, which is characterized by the intracellular insertion of microfilaments. If intermediate filaments are inserted in lieu of microfilaments, the resulting junction is referred to as a desmosome.

Perivascular endfeet
The specialized foot-processes of perivascular astrocytes that are closely apposed to the outer surface of brain microvessels, and have specialized functions in inducing and regulating the BBB.

Pericyte
A cell of mesodermal origin, and contractile-phagocytic phenotype, associated with the outer surface of capillaries.

The brain endothelial transporters that supply the brain with nutrients include the GLUT1 glucose carrier, several amino acid carriers (including LAT1, L-system for large neutral amino acids), and transporters for nucleosides, nucleobases and many other substances[10]. Several organic anion and cation transporters identified in other tissues and the choroid plexus are also proving to be expressed on the brain endothelium. Where compounds need to be moved against a concentration gradient, the energy may come from ATP (as in the ABC family of transporters, including P-glycoprotein (Pgp) and multidrug resistance-related proteins, MRPs), or the Na^+ gradient created by operation of the abluminal Na^+,K^+-ATPase. Some transporters (for example, GLUT1 and LAT1) are bidirectional, moving substrates down the concentration gradient, and can be present on both luminal and abluminal membranes, or predominantly on one. Quantification of GLUT1 expression on luminal and abluminal endothelial membranes is complicated by the fact that some antibodies do not recognize the transporter when the C-terminal is masked, as it may be in the luminal membrane[23]. Among the efflux transporters, Pgp is concentrated on the luminal membrane[24], whereas the Na^+-dependent transporters are generally abluminal, specialized for moving solutes out of the brain[25,26]. They include several Na^+-dependent glutamate transporters (excitatory amino acid transporters 1–3; EAAT1–3)[27], which move glutamate out of the brain against the large opposing concentration gradient (<1 μM in ISF compared with ~100 μM in plasma) (FIG. 2). The clear apical–basal polarity of brain endothelial cells noted above is hence reflected in their polarized transport function[20,28].

Induction of BBB properties

What causes the endothelium of blood vessels growing into the brain during development to become so specialized? It has been clear from the earliest histological studies that brain capillaries are surrounded by or closely associated with several cell types, including the perivascular endfeet of astrocytic glia, pericytes, microglia and neuronal processes (FIG. 2). In the larger vessels (arterioles, arteries and veins), smooth muscle forms a continuous layer, replacing pericytes[1]. Neuronal cell bodies are typically no more than ~10 μm from the nearest capillary[6]. These close cell–cell associations, particularly of astrocytes and brain capillaries, led to the suggestion that they could mediate the induction of the specific features of the barrier phenotype in the capillary endothelium of the brain[29].

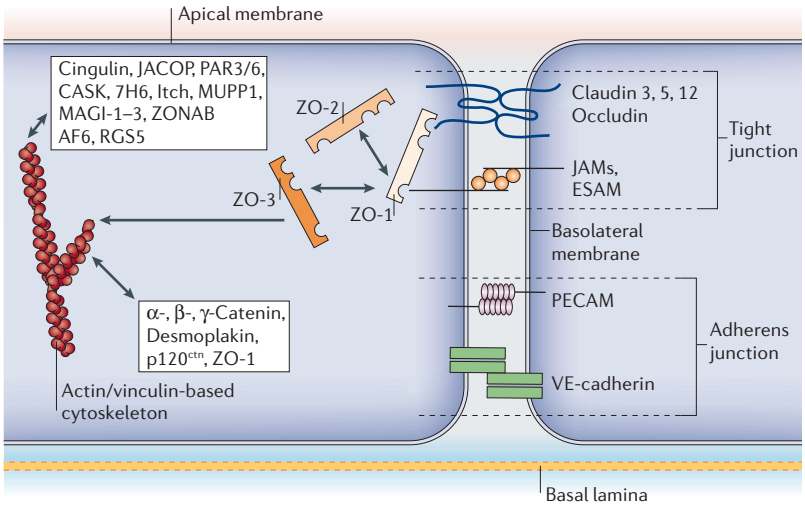

Figure 4 | **Molecular composition of endothelial tight junctions.** Simplified and incomplete scheme showing the molecular composition of endothelial tight junctions. Occludin and the claudins — proteins with four transmembrane domains and two extracellular loops — are the most important membranous components. The junctional adhesion molecules (JAMs) and the endothelial selective adhesion molecule (ESAM) are members of the immunoglobulin superfamily. Within the cytoplasm are many first-order adaptor proteins, including zonula occludens 1, 2 and 3 (ZO-1–3) and Ca^{2+}-dependent serine protein kinase (CASK), that bind to the intramembrane proteins. Among the second-order adaptor molecules, cingulin is important, and junction-associated coiled-coil protein (JACOP) may also be present. Signalling and regulatory proteins include multi-PDZ-protein 1 (MUPP1), the partitioning defective proteins 3 and 6 (PAR3/6), MAGI-1–3 (membrane-associated guanylate kinase with inverted orientation of protein–protein interaction domains), ZO-1-associated nucleic acid-binding protein (ZONAB), afadin (AF6), and regulator of G-protein signalling 5 (RGS5). All of these adaptor and regulatory/signalling proteins control the interaction of the membranous components with the actin/vinculin-based cytoskeleton. In epithelial cells, tight and adherens junctions are strictly separated from each other, but in endothelial cells these junctions are intermingled. The most important molecule of endothelial adherens junctions is vascular endothelial cadherin (VE-cadherin). In addition, the platelet–endothelial cell adhesion molecule (PECAM) mediates homophilic adhesion. The chief linker molecules between adherens junctions and the cytoskeleton are the catenins, with desmoplakin and p120 catenin ($p120^{ctn}$) also involved. Itch, E3 ubiquitin protein ligase. Modified, with permission, from REF. 20 © (2005) Wiley-VCH.

Orthogonal arrays of particles
(OAPs). The organized arrays (square lattice) of intramembranous particles detected by the freeze–fracture technique in certain astrocyte processes. First identified on the polarized endfeet on blood vessels and in the outer glial layer (glia limitans) below the pia, they have subsequently been shown to contain specific protein complexes held together by structural proteins.

Basal lamina
The extracellular matrix layer produced by the basal cell membrane, used as an anchoring and signalling site for cell–cell interactions.

Astrocytes show a number of different morphologies, depending on their location and association with other cell types. Of the ~11 distinct phenotypes that can be readily distinguished, 8 involve specific interactions with blood vessels[30]. There is now strong evidence, particularly from studies in cell culture, that astrocytes can upregulate many BBB features, leading to tighter tight junctions (physical barrier)[31,32], the expression and polarized localization of transporters, including Pgp[24] and GLUT1 (REF. 33) (transport barrier), and specialized enzyme systems (metabolic barrier)[9,34–36]. More recently, some of the other cell types present at the BBB, including pericytes, perivascular macrophages and neurons, have also been shown to contribute to barrier induction[37–43]. Given the complexity of the barrier properties of the BBB, and the anatomical relationships of the associated cells, it is not surprising to find synergistic inductive functions involving more than one cell type. For example, astrocytes are necessary for the correct association of endothelial cells and pericytes in tube-like structures *in vitro*[38], which suggests that interactions between the three cell types are also required for proper cerebral capillary differentiation *in vivo*.

The converse induction, in which brain endothelium enhances the growth and differentiation of associated astrocytes, has also been shown[44,45]. Indeed, upregulation of the endothelial enzyme γ-glutamyl transpeptidase (γGTP) involves a two-way induction with astrocytes[46], and co-culture results in the upregulation of antioxidant enzymes in both endothelial cells and astrocytes[47].

Specializations of astrocytic perivascular endfeet. Astrocytes are derived from ependymoglia of the developing neural tube, and retain some features of their original apical–basal polarity, together with more specific polarization of function in relation to particular cell–cell associations of the adult[28,30]. The perivascular endfeet of astrocytes, which are closely applied to the microvessel wall, show several specialized features characteristic of this location, including a high density of orthogonal arrays of particles (OAPs) containing the water channel aquaporin 4 (AQP4) and the Kir4.1 K^+ channel, which are involved in ion and volume regulation (see below). The OAPs/AQP4 polarity of astrocytes correlates with the expression of agrin, a heparin sulphate proteoglycan, on the basal lamina[11,48]. Agrin accumulates in brain microvessels at the time of BBB tightening, and is important for the integrity of the BBB[20]. The agrin splice variant Y0Z0 is a specific component of the endothelial basal lamina of CNS capillaries. Agrin is required for the segregation of AQP4 to the perivascular astrocytic endfeet, mediated by agrin binding to α-dystroglycan (a member of the dystrophin–dystroglycan complex, DDC), which couples to AQP through α1-syntrophin, another member of the DDC. α-Syntrophin also binds to Kir4.1, which explains the co-localization of Kir4.1 and AQP4. The precise localization of this complex array of membrane proteins in the astrocytic endfeet, anchored by agrin in the basal lamina, provides part of the evidence that this extracellular matrix makes an important contribution to the inductive influences between the endothelium and astrocytes.

Inducing factors. Astrocytes are able to secrete a range of chemical agents[9,28,36,49]. Several of these glia-derived factors, including transforming growth factor-β (TGFβ), glial-derived neurotrophic factor (GDNF)[50], basic fibroblast growth factor (bFGF) and angiopoetin 1 (ANG1, acting on the TIE2 endothelium-specific receptor tyrosine kinase 2), can induce aspects of the BBB phenotype in endothelial cells *in vitro*[51]. Conversely, endothelium-derived leukaemia inhibitory factor (LIF) has been shown to induce astrocytic differentiation[45]. The defects in BBB function in some neuropathologies, especially those that involve glia (see below), suggest that continuing induction during adult life is necessary for normal function.

Box 1 | **Agents modifying brain endothelial function and BBB tightness**

A number of chemical agents circulating in the plasma or secreted from cells associated with the blood–brain barrier (BBB) are capable of increasing brain endothelial permeability and impairing its transport and metabolic functions[56,68]. Other agents have the opposite effect, improving tightness and BBB function.

Agents that impair BBB function:

- Bradykinin, histamine, serotonin, glutamate.
- Purine nucleotides: ATP, ADP, AMP.
- Adenosine, platelet-activating factor.
- Phospholipase A2, arachidonic acid, prostaglandins, leukotrienes.
- Interleukins: IL-1α, IL-1β, IL-6.
- Tumour necrosis factor-α (TNFα), macrophage-inhibitory proteins MIP1 and MIP2.
- Complement-derived polypeptide C3a-desArg.
- Free radicals, nitric oxide.

Agents that cause barrier tightening and improved function:

- Steroids, elevated intracellular cyclic AMP, adrenomedullin and noradrenergic agents.

Modulation of BBB function

The term barrier suggests a relatively fixed structure, but it is now known that many (and possibly most) features of the BBB phenotype can be subject to change (modulation)[16]. Some of the first examples of modulation were found in extreme or pathological conditions. For example, opening of the BBB's tight junctions can occur in inflammation, contributing to brain oedema[52], and upregulation of GLUT1 transporter expression at the BBB is observed in starvation and hypoxia[53,54].

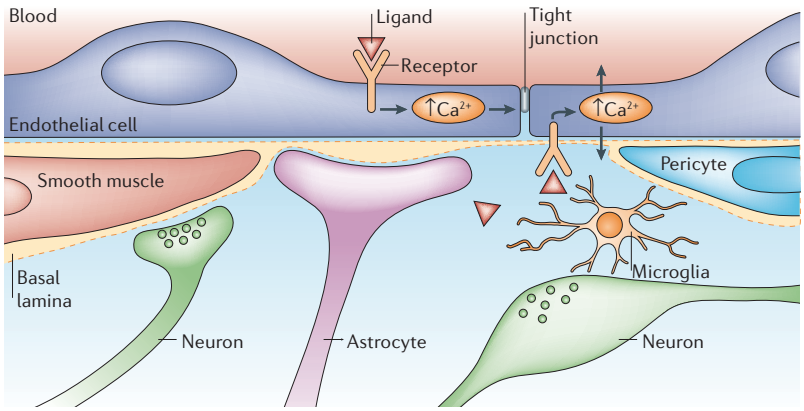

Figure 5 | **Complex cell–cell signalling at the blood–brain barrier.** A portion of a brain capillary wall, showing the main cell types present with the potential to signal to each other. Pericytes are enclosed within the endothelial basal lamina and form the closest associations with endothelium. The endfeet of astrocytic glial cells are apposed to the outer surface of the basal lamina. In the perivascular space are found microglia, the synaptic terminals and boutons of nerve fibres, and (in arterioles) smooth muscle cells. In the larger vessels, cells of the meninges form a perivascular cuff or sheath that projects down from the brain surface and demarcates the Virchow–Robin space (not shown). Agents such as ATP and histamine can influence endothelial function by ligand–receptor interaction, from the blood or the brain side. Some receptors are coupled to increases in the concentration of intracellular Ca^{2+}. The arrows indicate the ability of the endothelium to release substances to the blood or brain side after receptor activation, as part of their 'effector' function. Modified, with permission, from REF. 16 © (2005) Springer.

The protein leptin can enhance the transcytosis of the peptide urocortin across the BBB, with implications for the regulation of feeding[55]. Some of the inflammatory mediators that increase capillary permeability in the periphery (for example, histamine and bradykinin) also act on the brain endothelium, although in general higher concentrations are required and the effects are more localized and short-lasting in the brain[56]. There is some evidence that post-capillary venules are particularly vulnerable to opening by inflammatory mediators[57,58], which is relevant in pathologies such as multiple sclerosis (see below). BBB Pgp function is altered in several different conditions[59]. For example, upregulation of Pgp over hours to days can occur in oxidative stress[60] and on treatment with glutamate[61]. Pgp upregulation by steroids appears to involve transcriptional regulation via the nuclear pregnane X receptor (PXR)[62]. More rapid modulation (on a timescale of seconds) can be achieved by specific Pgp inhibitors and competitors[59], whereas endothelin 1 can cause functional inhibition[63].

These observations prompted characterization of the receptors present on the brain endothelium that are capable of mediating BBB modulation. Cultured brain endothelial cells and astrocytes express functional receptors for a high proportion of the agents that act as neurotransmitters and modulators in the brain[56,64] (BOX 1). As many of these are also released by astrocytes and endothelium, there is potential for complex signalling between cells in the neurovascular unit, including microglia and oligodendrocytes[65–67] (FIG. 5). Such rapid signalling (occurring over seconds to minutes), often mediated by agents with a short half-life, is distinct from the longer-term induction processes that are outlined above (hours to days), which generally involve the regulation of gene transcription and require protein synthesis.

The fact that agents released during normal neural activity can potentially influence both astrocytes and endothelium also raised the interesting possibility that signalling involving brain endothelium and glia could occur physiologically. There could be a physiological advantage in transiently 'opening' the BBB (tight junction modulation) — for example, triggered by histamine released from nerve terminals to allow the passage of growth factors and antibodies into the brain from plasma, or to 'sample' plasma composition[9]. Conversely, mechanisms for tightening the barrier could be important in conditions of stress or hypoxia: it is known that conditions in which intracellular cyclic AMP (cAMP) concentrations are increased can lead to increased TEER and upregulation of Pgp activity[68]. The transcription factor NF-κB can alter tight junction protein expression and hence regulate BBB permeability[69]. Several regulatory mechanisms influence the transport of glucose and amino acids by the brain endothelium[70]. In cultured brain endothelial cells, glucose transport can be increased by histamine and ATP, which could be part of a mechanism by which astrocytes sense neuronal firing and signal to the capillaries to supply more glucose, a form of neurobarrier coupling[71–73]. Indeed, brain endothelial glucose uptake has been shown to be

Box 2 | **Relationship between studies in cell culture and in brain slices**

Until recently, most studies of astrocyte–endothelial interaction have been done in cell culture, in which well-characterized cells can be grown together (or exposed to conditioned medium from the other cell type) to test hypotheses about blood–brain barrier (BBB) induction and modulation. During the past few years, a number of studies in brain slices and *in situ* have permitted experiments under conditions closer to those present *in vivo*. In particular, the brain slice contains differentiated cells in close to their normal relations with each other, overcoming some of the problems of de-differentiation and simplification that are involved in cell culture. However, the greater complexity of the slice makes it harder to resolve underlying mechanisms.

In situ, groups of astrocytes are coupled by gap junctions, forming clusters that define 'microdomains' of neural or glial function, with associated capillaries forming the boundaries[3]. In brain slices, ATP application mobilized cytosolic Ca^{2+} in perivascular endfeet, whereas electrical stimulation triggered Ca^{2+} waves spreading along the vessel wall[88]. Purinergic receptors (P_2Y_2 and P_2Y_4) were found on the astrocytes, but not on endothelium or pericytes, and the gap junction protein connexin 43 (CX43) was expressed in the astrocytic perivascular endfeet but not in endothelium. The conclusion was that the Ca^{2+} wave propagated along the syncytium of astrocytic endfeet, but did not continue into the endothelium. Zonta *et al.*[149] showed that astrocytes can detect the level of glutamate-dependent synaptic activity, then signal to adjacent cerebral vessels, causing vasodilation by a mechanism involving prostanoids. This was proposed as a new mechanism to couple neural activity to blood flow, but whether the effect involved endothelium was not clear.

Slice preparations offer promising possibilities for examining 'neurobarrier' modulation in close to physiological conditions, and for assessing the roles of the different cell types in the neurovascular unit in BBB modulation, testing some of the ideas generated from cell culture preparations[71,73].

enhanced by factors released from astrocytes exposed to hypoglycaemic conditions[74].

The signal transduction pathways involved in BBB modulation have been studied extensively. Several of the receptors found on brain endothelium and astrocytes cause an increase in intracellular Ca^{2+} when activated[75,76], and Ca^{2+}-mediated signalling is one mechanism by which CNS cells communicate with and modulate the activity of adjacent cells[77] (FIG. 5). The spread of calcium waves through the astrocytic syncytium, propagating at a rate of ~100 μm s^{-1}, can be triggered by activation of 5-hydroxytryptamine (5-HT, serotonin) or glutamate receptors, or mechanical stimulation[78,79]. Inositol-1,4,5-trisphosphate is small enough to diffuse through gap junctions and may mediate cell–cell spread of the wave[80], whereas local release of glutamate or ATP may signal to adjacent cells[81]. Studies in culture suggest that endothelial cells can also couple with intercellular gap junctions, and both release and respond to ATP, providing a possible means of propagating the signal along the capillary *in situ*[82]. Thus, the machinery is available for coordinating the activity of neurons, astrocytes and endothelium in and between neurovascular microdomains. Signalling interactions between microglia, astrocytes and neurons have also been observed in culture[66,83], and may become particularly important in pathological conditions.

What are the downstream consequences of such signalling? An increase in intracellular Ca^{2+} concentration can cause changes in a number of effector proteins of the brain endothelium through several signal transduction pathways, including phosphorylation of cytoskeletal proteins and tight junction opening[11,52]. Given the large repertoire of astrocyte-released agents[9,49], many of which have matching receptors on brain endothelium, a range of distinct and complex responses of the endothelium could be orchestrated. This would allow neuronal activity to be signalled to the endothelium, either directly or via astrocytes, resulting in modulation of the brain endothelium to increase its efficiency as a nutrient source and metabolic device[71,73]. As further examples of transmitter-mediated modulation are discovered, acting on membranes, membrane-associated protein complexes, junctional proteins, transporters and enzymes, the details of such coordination will become clearer. It has recently become possible to study signalling in the neurovascular unit in brain slices, which is a valuable way of testing the ideas generated from cell culture studies (BOX 2).

Ion and volume regulation at the BBB

We have seen that astrocytes occupy a strategic position between capillaries and neurons. Gap junctions between astrocyte processes allow them to communicate with each other, and other astrocyte processes are in contact with the endothelial cells of the capillaries that form the BBB[30] (FIG. 2). In normal brain activity, neurons release neurotransmitters and K^+, and take up Na^+, while glucose metabolism generates water at the rate of ~28 nl g^{-1} min^{-1} (REF. 84). The neurotransmitters and ions are generally recycled, whereas water must be removed from the brain and excreted. Astrocytes contribute to ionic, amino acid, neurotransmitter and water homeostasis of the brain in several ways, and astrocytes that form perivascular endfeet at the BBB have a particular role[85] in these processes.

An increase in extracellular K^+ around astroglial processes leads to K^+ entry and membrane depolarization, and the electrochemical gradient that is set up can lead to K^+ efflux at distant cell processes not experiencing the elevated K^+ concentration (the K^+ spatial buffer mechanism)[86]. The high density of appropriate K^+ channels on perivascular astrocytic endfeet (especially inwardly rectifying Kir4.1, and possibly including the Ca^{2+}-dependent *rSlo*K_{Ca} channels[87]) makes them well suited for spatial buffering, depositing the K^+ in the perivascular space. As the brain endothelium has a low K^+ permeability, the K^+ is not generally lost from the brain, but can be recycled (by reversal of the spatial

Box 3 | **Pathological states involving BBB breakdown or disorder**

Several pathologies of the CNS involve disturbance of blood–brain barrier (BBB) function, and, in many of these, astrocyte–endothelial cooperation is also abnormal.

Stroke

- Astrocytes secrete transforming growth factor-β (TGFβ), which downregulates brain capillary endothelial expression of fibrinolytic enzyme tissue plasminogen activator (tPA) and anticoagulant thrombomodulin (TM)[150].
- Proteolysis of the vascular basement membrane/matrix[151].
- Induction of aquaporin 4 (AQP4) mRNA and protein at BBB disruption[152].
- Decrease in BBB permeability after treatment with arginine vasopressin V1 receptor antagonist in a stroke model[153].

Trauma

- Bradykinin, a mediator of inflammation, is produced and stimulates production and release of interleukin-6 (IL-6) from astrocytes, which leads to opening of the BBB[102].

Infectious or inflammatory processes

Examples include bacterial infections, meningitis, encephalitis and sepsis.

- The bacterial protein lipopolysaccharide affects the permeability of BBB tight junctions. This is mediated by the production of free radicals, IL-6 and IL-1β[154].
- Interferon-β prevents BBB disruption[155].

Multiple sclerosis

- Breakdown of the BBB[97].
- Downregulation of laminin in the basement membrane[156].
- Selective loss of claudin 1/3 in experimental autoimmune encephalomyelitis[94].

HIV

- BBB tight junction disruption[157,158].

Alzheimer's disease

- Increased glucose transport, upregulation of glucose transporter GLUT1, altered agrin levels, upregulation of AQP4 expression[95,159].
- Accumulation of amyloid-β, a key neuropathological feature of Alzheimer's disease, by decreased levels of P-glycoprotein transporter expression[160].
- Altered cellular relations at the BBB, and changes in the basal lamina and amyloid-β clearance[100].

Parkinson's disease

- Dysfunction of the BBB by reduced efficacy of P-glycoprotein[101].

Epilepsy

- Transient BBB opening in epileptogenic foci, and upregulated expression of P-glycoprotein and other drug efflux transporters in astrocytes and endothelium[98,99].

Brain tumours

- Breakdown of the BBB[161,162].
- Downregulation of tight junction protein claudin 1/3; redistribution of astrocyte AQP4 and Kir4.1 (inwardly rectifying K^+ channel)[20,93,96].

Pain

- Inflammatory pain alters BBB tight junction protein expression and BBB permeability[108].

buffer) when neural activity ceases. Astrocytes can also take up K^+ through transporters, particularly the Na^+,K^+-ATPase and NKCC1 Na^+,K^+2Cl^- co-transporters. For both channel- and transporter-mediated K^+ uptake, the net ion gain results in osmotic water uptake and slight cell swelling; the high density of AQP4 water channels in perivascular astrocytic endfeet facilitates redistribution of this water. As the brain endothelium has low water permeability (little or no aquaporin)[88–90], it is likely that the excess metabolic water joins the ISF being secreted into the pericapillary space by the endothelium[5]. ISF outflow involves perivascular spaces around large vessels, and clearance routes either through the CSF or following alternative pathways to neck lymphatics.

Neurotransmitter recycling can also lead to local changes in ions and water. Glutamate is the major excitatory transmitter of the brain, and astrocyte processes surrounding synapses can take up glutamate through transport proteins (particularly EAAT1 and 2); the transport is Na^+-dependent and accompanied by net uptake of ions and water, again contributing to water clearance at the BBB[85]. Glutamate is converted to glutamine within the astrocyte and recycled to the neurons. The slight astrocytic cell swelling that accompanies neuronal activity, resulting from activation by glutamate or ion uptake, leads to several cellular mechanisms that contribute to the recovery of ionic balance and cell volume, some of which involve elevated intracellular Ca^{2+} concentration[66,91,92]. Hence, there are many links between the signalling and regulatory processes that occur in the neurovascular unit.

BBB changes in pathology

In a number of pathologies, the function of the BBB is altered (BOX 3), and several disorders appear to involve disturbances of endothelial–glial interaction. Thus, the capillaries of many glial tumours are more leaky than those of normal brain tissue, either as a result of a lack of inductive factors, or owing to the release of permeability factors such as vascular endothelial growth factor (VEGF). Moreover, the tight junction protein claudin 1/3 is downregulated in some brain tumours[93,94].

In BBB disruption, agrin is lost from the abluminal surface of the brain endothelial cells adjacent to astrocytic endfeet[11]; this may contribute to BBB damage in Alzheimer's disease[95], and to the redistribution of astrocytic AQP4 in glioblastomas[96]. Astrocytic AQP4 expression is upregulated in brain oedema triggered by BBB breakdown. Such upregulation could be adaptive in helping to clear the accumulating fluid, but the associated cell swelling would tend to exacerbate the problem under extreme conditions. Indeed, $AQP4^{-/-}$ mice show protection against ischaemic brain oedema[48]. Some chronic neuropathologies such as multiple sclerosis may involve an early phase of BBB disturbance (involving the downregulation of claudin 1/3 (REF. 11)) that precedes neuronal damage, which suggests that vascular damage can lead to secondary neuronal disorder[97].

In epilepsy, the normal pattern of brain ABC transporter expression may change, with upregulation of Pgp on astrocytes and brain endothelium[98,99]; this may be an adaptive response to barrier opening (and hence a less efficient BBB), which is often seen during seizure activity.

In animal models of Alzheimer's disease, amyloid-β (Aβ) accumulation is often first seen in the neighbourhood of blood vessels, with toxicity on endothelium and astrocytes observed before significant neuronal loss[1];

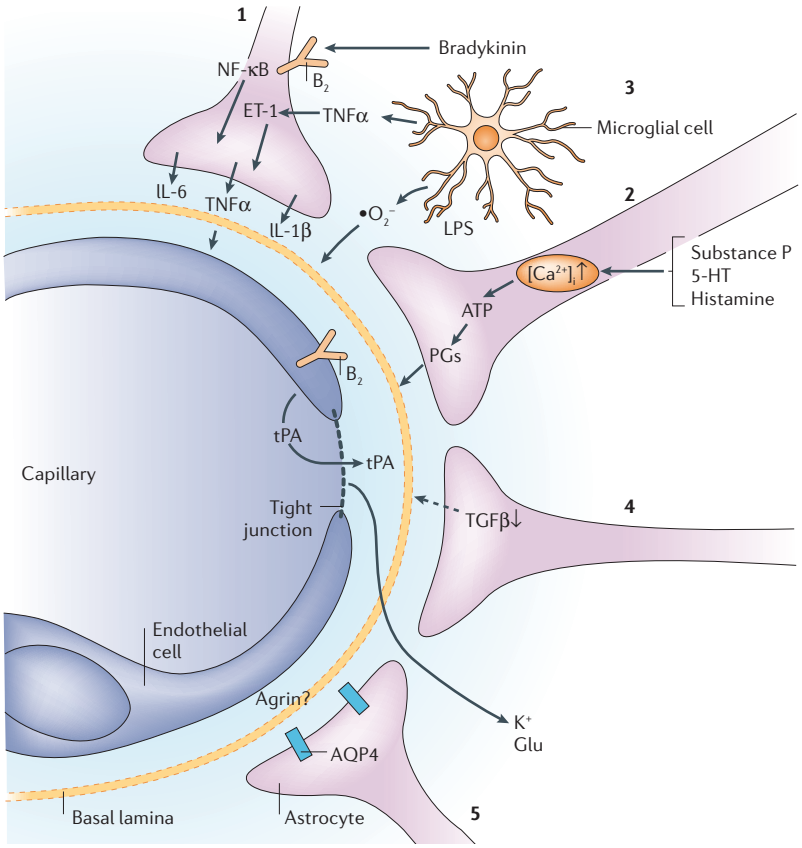

Figure 6 | **Astroglial–endothelial signalling under pathological conditions.** Examples of astroglial–endothelial signalling in infection or inflammation, stroke or trauma, leading to opening of the blood–brain barrier (BBB) and disturbance of brain function. bradykinin, produced during inflammation in stroke or brain trauma, acts on endothelial and astroglial bradykinin B_2 receptors, leading to an increase in the concentration of intracellular Ca^{2+}. In astrocytes, this can trigger the production of interleukin-6 (IL-6) through activation of nuclear factor-κB (NF-κB) (**1**). Bradykinin, substance P, 5-hydroxytryptamine (5-HT, serotonin) and histamine acting on astrocytes can lead to the formation of ATP and prostaglandins (PGs), with effects on vascular tone and endothelial permeability (**2**) by mechanisms that are known to involve endothelium. Lipopolysaccharide (LPS), formed in infections, leads to the release from microglia of tumour necrosis factor-α (TNFα), IL-1β and reactive oxygen species (including $O_2^{•-}$), all of which have the ability to open the BBB (**3**). Astrocytes downregulate tissue plasminogen activator (tPA) production via transforming growth factor-β (TGFβ), but there is still sufficient tPA to open the BBB, leading to an influx of tPA from the blood (**4**). Following disruption of the BBB involving a decrease in agrin expression, K^+ and glutamate (Glu) from the blood can reach the brain extracellular space. Aquaporin 4 (AQP4) is upregulated on the astroglial endfeet, leading to astroglial swelling (**5**). ET1, endothelin 1.

disturbances of CNS homeostasis as a result of barrier deficiencies could contribute to and exacerbate the later neuropathology[100]. Recently, Kortekaas *et al.*[101] showed an elevated uptake of the Pgp substrate [^{11}C]verapamil using positron emission tomography (PET) in the midbrain of patients with Parkinson's disease, which is consistent with disturbed Pgp function in the BBB.

The ability of agents released during inflammation to increase the permeability of the brain endothelium may depend on associated cell types (FIG. 6). Thus, as well as acting on endothelial bradykinin B_2 receptors to raise intracellular Ca^{2+} concentrations and open tight junctions, bradykinin can activate NF-κB in astrocytes, leading to the release of interleukin-6 (IL-6), which can amplify the effect by acting back on the endothelium[102]. Tumour necrosis factor-α (TNFα) can increase BBB permeability by direct actions on the endothelium[103] and indirect effects involving endothelial endothelin 1 production and IL-1β release from astrocytes, in a complex immunoregulatory loop[104]. Systemic infection can exacerbate CNS inflammatory pathologies such as multiple sclerosis by several mechanisms, including activation of already primed central macrophages, with some mechanisms effective even with an intact BBB[105]. Indeed, the ability of the BBB to transport cytokines may contribute to the link between central and peripheral disease[106].

It has recently been proposed that activated astrocytes and microglial cells could maintain neuropathic pain[107]. As astrocytes have extensive gap junctional connectivity and form glial networks, it has been suggested that glia may be involved in the spreading of pain sensation. In injury, several substances are released from central and peripheral neurons, connective tissue cells and blood cells. Many of these substances, such as substance P, calcitonin gene-related peptide (CGRP), serotonin, histamine and ATP, can affect the BBB from both the blood and the nervous tissue sides. For example, the release of IL-1β leads to a decreased concentration or altered localization of the tight junction protein occludin, and increased BBB permeability. TNFα, histamine and interferon-γ released in inflammatory pain can also cause changes in brain endothelial permeability[108].

The involvement of microglia in signalling within the pathological neurovascular unit has been mentioned above[66,67]. It is possible that damage to the endothelium and basal lamina allows expression of endothelial receptors that are normally downregulated (for example, receptors for nucleotides such as ATP), opening new communication loops between endothelium, pericytes, astrocytes and microglia that are important in barrier repair.

Targeting the BBB to fight disease

The BBB as a therapeutic target. We have seen how information on the routes across the BBB (FIG. 3) needs to be taken into account in developing drug delivery strategies to target sites in the CNS in treating neural disorders. Given the evidence for involvement of BBB damage as an early event in many neurological conditions, it is not surprising that there is growing interest in the BBB as a therapeutic target in its own right[109–112]. The underlying logic is that if BBB dysfunction can be reduced, halted or reversed, this could be valuable therapy in conditions in which neuronal damage is secondary to, or exacerbated by, BBB damage. Steroids such as dexamethasone are widely used to reduce inflammation, and are an accepted treatment for brain oedema[113]. It is now known that dexamethasone can improve barrier function not only by increasing the tightness of the brain endothelial tight junctions[114], but also by upregulating BBB Pgp[59]. Ca^{2+} channel blockers hold promise for reducing brain

damage in hypoxia[115] and hypertension[116], by reducing the Ca^{2+}-mediated increase in BBB permeability. Local brain hypothermia reduces BBB damage and oedema following intracerebral haemorrhage, reducing the severity of neuronal damage[117,118]. A pan-caspase inhibitor given intraperitoneally reduced brain endothelial permeability and brain oedema after subarachnoid haemorrhage[119]. This shift in emphasis from the rescue of neurons to treatment of the BBB means that the brain endothelium itself becomes a target for drug action.

Techniques such as differential display are already being used to establish which genes expressed by the brain endothelium are upregulated in disease[120], and some of these could be targets for therapy. However, caution is needed because many of the changes will be part of the defensive response of the endothelium, and the same agents that are destructive at certain phases of disease (for example, some cytokines, such as TNFα) may have important protective actions at earlier stages or at lower concentrations. Moreover, interactions between agents, both inhibitory and potentiating, make it difficult to devise strategies targeted to individual substances or receptors.

VEGF, which increases brain endothelial permeability when given intravenously, is neuroprotective and reduces BBB leakage after ischaemia when given intraventricularly[121], which indicates that it has either a differential action on the apical versus the basal side of the brain endothelium, or an ability to act through other cells on the brain side. As the role of cell–cell interactions in the neurovascular unit becomes better understood, other cells associated with the BBB may also become useful therapeutic targets[122].

Opening the BBB for therapeutic purposes. Most of the treatments mentioned so far are designed to seal up and improve the transport function of a BBB made leaky by disease. The opposite approach — deliberately opening the tight junctions of the brain endothelium to facilitate drug delivery to the brain — is the subject of an extensive literature[123]. From the discussion above (role of BBB in brain homeostasis), it is clear that therapeutic BBB opening needs to be kept as brief as is practical to reduce oedema and other side effects. Opening of the BBB using intracarotid infusion of hyperosmolar solutions has had some success in increasing drug delivery to tumours[123,124]; the mechanism for the opening effect may involve phosphorylation of the adherens junction protein β-catenin[125]. Attempts to produce controlled BBB opening using analogues of inflammatory mediators such as bradykinin (Cereport, also called RMP-7) have shown promise in animal studies but not reproducible efficacy in clinical trials[126]. Improvements in both types of approach are being pursued[125].

Protective strategies at the BBB. There is growing evidence that maintaining endothelial health can reduce the incidence or severity of systemic vascular disease in at-risk individuals, in conditions such as atherosclerosis, lupus and diabetes[127–130]. Moderate exercise[131,132], and a diet rich in fish oils[133,134], fruit[135,136], soy[137,138], vitamins C and E[139–141], garlic[127] and red wine[142] may be beneficial[143].

Although less specifically studied, protection of the BBB has the potential to delay or prevent the development of chronic neurodegeneration. Indeed, many plant-derived compounds, such as flavonoids, and other polyphenolic agents that are being investigated as neuroprotectants[144] also have beneficial effects on the endothelium[145,146]. The cytokine erythropoietin (a major regulator of erythropoiesis) is protective against brain injury *in vivo*, and protects cultured neurons against toxicity[147]; moreover, it protects brain endothelium against VEGF-induced permeability by reducing the level of endothelial nitric oxidase synthase (eNOS) and restoring junctional proteins[148].

Would it be possible to go further, and alter the properties of a largely healthy BBB to improve its ability to protect the brain? For example, would upregulation of BBB Pgp or other transporters be beneficial? So far, this is a relatively unexplored field. It has potential, but would require careful consideration of the context, within the neurovascular unit and the whole body. For example, we do not yet know whether endogenous regulation of the expression and activity of BBB transporters maintains a delicate balance between the needs to admit key substances and to keep out others — disturbing such a balance could have unpredictable side effects.

Concluding remarks and future perspectives

We have shown that specific interactions between brain endothelium and astrocytes within neurovascular units can influence the BBB under both physiological and pathological conditions. Mutual induction helps to establish the differentiated phenotype of both the cells involved in the association, upregulating barrier properties in the endothelium, and specific features of the astrocytic endfeet, including those involved in ionic and water regulation. Several pathologies that result in neural damage and degeneration may show an early phase involving BBB disorder, so early treatment of the barrier could reduce the severity of neuropathological symptoms and facilitate recovery. Even better, prophylactic treatment to maintain a healthy BBB has the potential to delay the onset of neurodegeneration. In the future, detailed investigation of the mechanisms involved in endothelial–astrocytic interaction could help in the design of therapies targeted at specific features necessary for BBB function. There will be several challenges. Improved semi-intact preparations (brain slices, *in situ* preparations) are needed to bridge the gap between studies in cell culture and those using animal models. We will need a better understanding of cellular proteomics and metabolism to devise more targeted therapies for the BBB. We need better ways of imaging and monitoring functions of the living brain to validate human treatments. And, finally, differences in genetic make-up, gender, age and environment can affect the BBB in subtle ways, so successful treatment may depend on individual microprofiling and the development of 'personalized medicine'.

1. Iadecola, C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nature Rev. Neurosci.* **5**, 347–360 (2004).
2. Anderson, C. M. & Nedergaard, M. Astrocyte-mediated control of cerebral microcirculation. *Trends Neurosci.* **26**, 340–344 (2003).
3. Nedergaard, M., Ransom, B. & Goldman, S. A. New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci.* **26**, 523–530 (2003).
4. Davson, H. & Segal, M. B. *Physiology of the CSF and Blood–Brain Barriers* (CRC, Boca Raton, USA, 1995).
5. Abbott, N. J. Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. *Neurochem. Int.* **45**, 545–552 (2004).
6. Schlageter, K. E., Molnar, P., Lapin, G. D. & Groothuis, D. R. Microvessel organization and structure in experimental brain tumors: microvessel populations with distinctive structural and functional properties. *Microvasc. Res.* **58**, 312–328 (1999).
7. Risau, W. & Wolburg, H. Development of blood–brain barrier. *Trends Neurosci.* **13**, 174–178 (1990).
8. Abbott, N. J. & Romero, I. A. Transporting therapeutics across the blood–brain barrier. *Mol. Med. Today* **2**, 106–113 (1996).
9. Abbott, N. J. Astrocyte–endothelial interactions and blood–brain barrier permeability. *J. Anat.* **200**, 629–638 (2002).
10. Begley, D. J. & Brightman, M. W. Structural and functional aspects of the blood–brain barrier. *Prog. Drug Res.* **61**, 40–78 (2003).
11. Wolburg, H. & Lippoldt, A. Tight junctions of the blood–brain barrier: development, composition and regulation. *Vasc. Pharmacol.* **38**, 323–337 (2002).
12. Hawkins, B. T. & Davis, T. P. The blood–brain barrier/neurovascular unit in health and disease. *Pharmacol. Rev.* **57**, 173–185 (2005).
13. El-Bacha, R. S. & Minn, A. Drug metabolizing enzymes in cerebrovascular endothelial cells afford a metabolic protection to the brain. *Cell. Mol. Biol.* **45**, 15–23 (1999).
14. Pardridge, W. M. Blood–brain barrier drug targeting: the future of brain drug development. *Mol. Interv.* **3**, 90–105 (2003).
15. Ge, S., Song, L. & Pachter, J. S. Where is the blood–brain barrier…really? *J. Neurosci. Res.* **79**, 421–427 (2005).
16. Abbott, N. J. Dynamics of CNS barriers: evolution, differentiation and modulation. *Cell. Mol. Neurobiol.* **25**, 5–23 (2005).
17. Cserr, H. F. & Bundgaard, M. Blood–brain interfaces in vertebrates: a comparative approach. *Am. J. Physiol.* **246**, R277–R288 (1984).
18. Brown, P. D., Davies, S. L., Speake, T. & Millar, I. D. Molecular mechanisms of cerebrospinal fluid production. *Neuroscience* **129**, 957–970 (2004).
19. Chodobski, A. & Szmydynger-Chodobska, J. Choroid plexus: target for polypeptides and site of their synthesis. *Microsc. Res. Tech.* **52**, 65–82 (2001).
20. Wolburg, H. in *Blood–Brain Interfaces — from Ontogeny to Artificial Barriers* (eds Dermietzel, R., Spray, D. & Nedergaard, M.) 77–107 (Wiley-VCH, Weinheim, Germany, in the press).
21. Butt, A. M., Jones, H. C. & Abbott, N. J. Electrical resistance across the blood–brain barrier in anaesthetized rats: a developmental study. *J. Physiol. (Lond.)* **429**, 47–62 (1990).
22. Yu, A. *et al.* Knockdown of occludin expression leads to diverse phenotypic alterations in epithelial cells. *Am. J. Physiol. Cell Physiol.* **288**, 1231–1241 (2005).
23. Simpson, I. A., Vanucci, S., DeJoseph, M. R. & Hawkins, R. A. Glucose transporter asymmetries in the bovine blood–brain barrier. *J. Biol. Chem.* **276**, 12725–12729 (2001).
24. Schinkel, A. H. P-glycoprotein, a gatekeeper in the blood–brain barrier. *Adv. Drug Deliv. Rev.* **36**, 179–194 (1999).
25. Hawkins, R. A., Peterson, D. R. & Vina, J. R. The complementary membranes forming the blood–brain barrier. *IUBMB Life* **54**, 101–107 (2002).
26. O'Kane, R. & Hawkins, R. A. Na^+-dependent transport of large neutral amino acids occurs at the abluminal membrane of the blood–brain barrier. *Am. J. Physiol. Endocrinol. Metab.* **285**, E1167–E1173 (2003).
27. O'Kane, R. L., Martinez-Lopez, I., DeJoseph, M. R., Vina, J. R. & Hawkins, R. A. Na^+-dependent glutamate transporters (EAAT1, EAAT2, and EAAT3) of the blood–brain barrier. *J. Biol. Chem.* **274**, 31891–31895 (1999).
28. Abbott, N. J. in *Blood–Brain Interfaces — From Ontology to Artificial Barriers* (eds Dermietzel, R., Spray, D. & Nedergaard, M.) 189–208 (Wiley-VCH, Weinheim, Germany, in the press).
29. Davson, H. & Oldendorf, W. H. Transport in the central nervous system. *Proc. R. Soc. Med.* **60**, 326–328 (1967).
30. Reichenbach, A. & Wolburg, H. in *Neuroglia* 2nd edn (eds Kettemann, H. & Ransom, B. R.) 19–35 (Oxford Univ. Press, New York, 2004).
31. Dehouck, M.-P., Meresse, S., Delorme, P., Fruchart, J. C. & Cecchelli, R. An easier, reproducible, and mass-production method to study the blood–brain barrier *in vitro*. *J. Neurochem.* **54**, 1798–1801 (1990).
One of the first papers to describe a reliable method for generating an endothelial–astrocyte co-culture model of the BBB tight enough for study of permeability and transport. The model has since been successful in functional and mechanistic studies.
32. Rubin, L. L. *et al.* A cell culture model of the blood–brain barrier. *J. Cell Biol.* **115**, 1725–1735 (1991).
33. McAllister, M. S. *et al.* Mechanisms of glucose transport at the blood–brain barrier: an *in vitro* study. *Brain Res.* **409**, 20–30 (2001).
Uses high-resolution confocal microscopy and permeability studies to show how perivascular astrocytes influence glucose transport by the brain endothelium.
34. Hayashi, Y. *et al.* Induction of various blood–brain barrier properties in non-neuronal endothelial cells by close apposition to co-cultured astrocytes. *Glia* **19**, 13–26 (1997).
35. Sobue, K. *et al.* Induction of blood–brain barrier properties in immortalized bovine brain endothelial cells by astrocytic factors. *Neurosci. Res.* **35**, 155–164 (1999).
36. Haseloff, R. F., Blasig, I. E., Bauer, H.-C. & Bauer, H. In search of the astrocytic factor(s) modulating blood–brain barrier functions in brain capillary endothelial cells *in vitro*. *Cell. Mol. Neurobiol.* **25**, 25–39 (2005).
37. Duport, S. *et al.* An *in vitro* blood–brain barrier model: cocultures between endothelial cells and organotypic brain slice cultures. *Proc. Natl Acad. Sci. USA* **95**, 1840–1845 (1998).
38. Ramsauer, M., Krause, D. & Dermietzel, R. Angiogenesis of the blood–brain barrier *in vitro* and the function of cerebral pericytes. *FASEB J.* **16**, 1274–1276 (2002).
One of the first papers to study the complex interactions between endothelium, astrocytes and pericytes *in vitro*, giving insights into the development and maintenance of the neurovascular unit.
39. Zenker, D., Begley, D. J., Bratzke, H., Rübsamen-Waigmann, H. & von Briesen, H. Human blood-derived macrophages enhance barrier function of cultured primary bovine and human brain capillary endothelial cells. *J. Physiol. (Lond.)* **551**, 1023–1032 (2003).
40. Schiera, G. *et al.* Synergistic effects of neurons and astrocytes on the differentiation of brain capillary endothelial cells in culture. *J. Cell. Mol. Med.* **7**, 165–170 (2003).
41. Berezowski, V., Landry, C., Dehouck, M. P., Cecchelli, R. & Fenart, L. Contribution of glial cells and pericytes to the mRNA profiles of P-glycoprotein and multidrug resistance-associated proteins in an *in vitro* model of the blood–brain barrier. *Brain Res.* **1018**, 1–9 (2004).
42. Hori, S., Ohtsuki, S., Hosoya, K., Nakashima, E. & Terasaki, T. A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation *in vitro*. *J. Neurochem.* **89**, 503–513 (2004).
43. Dohgu, S. *et al.* Brain pericytes contribute to the induction and up-regulation of blood–brain barrier functions through transforming growth factor-β production. *Brain Res.* **1038**, 208–215 (2005).
44. Estrada, C., Bready, J. V., Berliner, J. A., Pardridge, W. M. & Cancilla, P. A. Astrocyte growth stimulation by a soluble factor produced by cerebral endothelial cells *in vitro*. *J. Neuropathol. Exp. Neurol.* **49**, 539–549 (1990).
45. Mi, H., Haeberle, H. & Barres, B. A. Induction of astrocyte differentiation by endothelial cells. *J. Neurosci.* **21**, 1538–1547 (2001).
An elegant study that used 'panning' to separate cell types from the optic nerve, showing convincingly that endothelium-derived LIF induces astrocyte differentiation.
46. Mizuguchi, H., Utoguchi, N. & Mayumi, T. Preparation of glial cell extracellular matrix: a novel method to analyze glial–endothelial interaction. *Brain Res. Brain Res. Protoc.* **1**, 339–343 (1997).
47. Schroeter, M. L. *et al.* Astrocytes enhance radical defence in capillary endothelial cells constituting the blood–brain barrier. *FEBS Lett.* **449**, 241–244 (1999).
This co-culture study shows clearly the 'mutual induction' that astrocytes and endothelial cells exert on each other — free radical defence enzymes are upregulated in both cell types when they are grown together.
48. Verkman, A. S. Aquaporin water channels and endothelial cell function. *J. Anat.* **200**, 617–627 (2002).
49. Garcia-Segura, L. M. & McCarthy, M. M. Minireview: role of glia in neuroendocrine function. *Endocrinology* **145**, 1082–1086 (2004).
50. Igarashi, Y. *et al.* Glial cell line-derived neurotrophic factor induces barrier function of endothelial cells forming the blood–brain barrier. *Biochem. Biophys. Res. Commun.* **261**, 108–112 (1999).
51. Lee, S.-W. *et al.* SSeCKS regulates angiogenesis and tight junction formation in blood–brain barrier. *Nature Med.* **9**, 900–906 (2003).
One of the most elegant and comprehensive studies of astrocyte–endothelial induction *in vitro*, revealing the novel role of Src-suppressor C-kinase substrate (SSeCKs) and angiopoetin 1.
52. Huber, J. D., Egleton, R. D. & Davis, T. P. Molecular physiology and pathophysiology of tight junctions in the blood–brain barrier. *Trends Neurosci.* **24**, 719–725 (2001).
53. Drewes, L. R. in *Introduction to the Blood–Brain Barrier — Methodology, Biology and Pathology* (ed. Pardridge, W. M.) 165–174 (Cambridge Univ. Press, Cambridge, UK, 1998).
54. Boado, R. J. & Pardridge, W. M. Glucose deprivation and hypoxia increase the expression of the GLUT-1 glucose transporter via a specific mRNA *cis*-acting regulatory element. *J. Neurochem.* **80**, 552–554 (2002).
55. Pan, W., Akerstrom, V., Zhang, J., Pejovic, V. & Kastin, A. J. Modulation of feeding-related peptide/protein signals by the blood–brain barrier. *J. Neurochem.* **90**, 455–461 (2004).
56. Abbott, N. J. Inflammatory mediators and modulation of blood–brain barrier permeability. *Cell. Mol. Neurobiol.* **20**, 131–147 (2000).
57. Webb, A. A. & Muir, G. D. The blood–brain barrier and its role in inflammation. *J. Vet. Intern. Med.* **14**, 399–411 (2000).
58. Tonra, J. R. Cerebellar susceptibility to experimental autoimmune encephalomyelitis in SJL/J mice: potential interaction of immunology with vascular anatomy. *Cerebellum* **1**, 57–68 (2002).
59. Bauer, B., Hartz, A. M., Fricker, G. & Miller, D. S. Modulation of p-glycoprotein transport function at the blood–brain barrier. *Exp. Biol. Med.* **230**, 118–127 (2005).
60. Nwaozuzu, O. M., Sellers, L. A. & Barrand, M. A. Signalling pathways influencing basal and H_2O_2-induced P-glycoprotein expression in endothelial cells derived from the blood–brain barrier. *J. Neurochem.* **87**, 1043–1051 (2003).
61. Zhu, H. J. & Liu, G. Q. Glutamate up-regulates P-glycoprotein expression in rat brain microvessel endothelial cells by an NMDA receptor-mediated mechanism. *Life Sci.* **75**, 1313–1322 (2004).
62. Bauer, B., Hartz, A. M. S., Fricker, G. & Miller, D. S. Pregnane X receptor up-regulation of P-glycoprotein expression and transport function at the blood–brain barrier. *Mol. Pharmacol.* **66**, 413–419 (2004).
Evidence that the nuclear PXR can upregulate Pgp function at the BBB, providing a mechanism for a number of long-term modulations of significance in physiology and pathology.
63. Hartz, A. M. S., Bauer, B., Fricker, G. & Miller, D. S. Rapid regulation of P-glycoprotein at the blood–brain barrier by endothelin-1. *Mol. Pharmacol.* **66**, 387–394 (2004).
Complementary to reference 62, this paper shows short-term modulation of Pgp by a signalling molecule released within the neurovascular unit.
64. Hansson, E. & Rönnbäck, L. Astrocytic receptors and second messenger systems. *Adv. Mol. Cell Biol.* **31**, 475–501 (2004).

65. Hansson, E. & Rönnbäck, L. Astrocytes in glutamate neurotransmission. *FASEB J.* **9**, 343–350 (1995).
66. Hansson, E. & Rönnbäck, L. Glial neuronal signalling in the central nervous system. *FASEB J.* **17**, 341–348 (2003).
67. Andersson, A., Rönnbäck, L. & Hansson, E. Lactate induces tumour necrosis factor-α and interleukin-6 release in microglial and astroglial enriched primary cultures. *J. Neurochem.* **93**, 1327–1333 (2005).
68. Kis, B. *et al.* Adrenomedullin regulates blood–brain barrier functions *in vitro*. *Neuroreport* **12**, 4139–4142 (2001).
69. Brown, R. C., Mark, K. S., Egleton, R. D. & Davis, T. P. Protection against hypoxia-induced increase in blood–brain barrier permeability: role of tight junction proteins and NFκB. *J. Cell Sci.* **116**, 693–700 (2003).
70. Mann, G. E., Yudilevich, D. L. & Sobrevia, L. Regulation of amino acid and glucose transport in endothelial and smooth muscle cells. *Physiol. Rev.* **83**, 183–252 (2003).
71. Braet, K., Cabooter, L., Paemeleire, K. & Leybaert, L. Calcium signal communication in the central nervous system. *Biol. Cell* **96**, 79–91 (2004).
72. Leybaert, L., Cabooter, L. & Braet, K. Calcium signal communication between glial and vascular brain cells. *Acta Neurol. Belg.* **104**, 51–56 (2004).
73. Leybaert, L. Neurobarrier coupling in the brain: a partner of neurovascular and neurometabolic coupling? *J. Cereb. Blood Flow Metab*. **25**, 2–16 (2005).
74. Régina, A. *et al.* Factor(s) released by glucose-deprived astrocytes enhance glucose transporter expression and activity in rat brain endothelial cells. *Biochim. Biophys. Acta* **1540**, 233–242 (2001).
One of the first papers to show that the metabolic status of astrocytes affects the way they influence the brain endothelium, which is of relevance in ischaemia and starvation.
75. Abbott, N. J. in *Introduction to the Blood–Brain Barrier: Methodology and Biology* (ed. Pardridge, W. M.) 345–353 (Cambridge Univ. Press, Cambridge, UK, 1998).
76. Muyderman, H. *et al.* α_1-Adrenergic modulation of metabotropic glutamate receptor-induced calcium oscillations and glutamate release in astrocytes. *J. Biol. Chem.* **276**, 46504–46514 (2001).
77. Pasti, L., Volterra, A., Pozzan, T. & Carmignoto, G. Intracellular calcium oscillations in astrocytes: a highly plastic, bidirectional form of communication between neurons and astrocytes *in situ*. *J. Neurosci.* **17**, 7817–7830 (1997).
78. Cornell-Bell, A. H., Finkbeiner, S. M., Cooper, M. S. & Smith, S. J. Glutamate induces calcium waves in cultured astrocytes: long-range glial signalling. *Science* **247**, 470–473 (1990).
79. Blomstrand, F. *et al.* 5-Hydroxytryptamine and glutamate modulate velocity and extent of intercellular calcium signalling in hippocampal astroglial cells in primary cultures. *Neuroscience* **88**, 1241–1253 (1999).
80. Sneyd, J. *et al.* A model for the propagation of intercellular calcium waves. *Am. J. Physiol.* **266**, C293–C302 (1994).
81. Cotrina, M. L. *et al.* Connexins regulate calcium signaling by controlling ATP release. *Proc. Natl Acad. Sci. USA* **95**, 15735–15740 (1998).
82. Paemeleire, K. & Leybaert, L. ATP-dependent astrocyte–endothelial calcium signalling following mechanical damage to a single astrocyte in astrocyte–endothelial co-cultures. *J. Neurotrauma* **17**, 345–358 (2000).
One of the first papers to investigate the mechanisms that underlie rapid astrocyte–endothelial signalling, using cultured cells. It is now becoming possible to do this kind of experiment in brain slices.
83. Bezzi, P. *et al.* CXCR4-activated astrocyte glutamate release via TNFα: amplification by microglia triggers neurotoxicity. *Nature Neurosci.* **4**, 702–710 (2001).
84. Rapoport, S. I. *Blood–Brain Barrier in Physiology and Medicine* (Raven, New York, USA, 1976).
85. Simard, M. & Nedergaard, M. The neurobiology of glia in the context of water and ion homeostasis. *Neuroscience* **129**, 877–896 (2004).
86. Kofuji, P. & Newman, E. A. Potassium buffering in the central nervous system. *Neuroscience* **129**, 1045–1056 (2004).
87. Price, D. L., Ludwig, J. W., Mi, H., Schwarz, T. L. & Ellisman, M. H. Distribution of *rSlo* Ca^{2+}-activated K^+ channels in rat astrocyte perivascular endfeet. *Brain Res.* **956**, 183–193 (2002).
88. Simard, M., Arcuino, G., Takano, T., Liu, Q. S. & Nedergaard, M. Signaling at the gliovascular interface. *J. Neurosci.* **23**, 9254–9262 (2003).
89. Amiry-Moghaddam, M. & Ottersen, O. P. The molecular basis of water transport in the brain. *Nature Rev. Neurosci.* **4**, 991–1001 (2003).
90. Dolman, D., Drndarski, S., Abbott, N. J. & Rattray, M. Induction of aquaporin 1 but not aquaporin 4 messenger RNA in rat primary brain microvessel endothelial cells in culture. *J. Neurochem.* **93**, 825–833 (2005).
91. Hansson, E. Metabotropic glutamate receptor activation induces astroglial swelling. *J. Biol. Chem.* **269**, 21955–21961 (1994).
92. Hansson, E., Johansson, B. B., Westergren, I. & Rönnbäck, L. Glutamate-induced swelling of single astroglial cells in primary culture. *Neuroscience* **63**, 1057–1066 (1994).
93. Liebner, S. *et al.* Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme. *Acta Neuropathol.* **100**, 323–331 (2000).
94. Wolburg, H. *et al.* Localization of claudin-3 in tight junctions of the blood–brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme. *Acta Neuropathol.* **105**, 586–592 (2003).
95. Berzin, T. M. *et al.* Agrin and microvascular damage in Alzheimer's disease. *Neurobiol. Aging* **21**, 349–355 (2000).
96. Warth, A., Kröger, S. & Wolburg, H. Redistribution of aquaporin-4 in human glioblastoma correlates with loss of agrin immunoreactivity from brain capillary basal laminae. *Acta Neuropathol.* **107**, 311–318 (2004).
Shows clearly the importance of the extracellular matrix in providing the scaffold for the ordering of proteins important in the function of astrocytic perivascular endfeet, and its disruption in pathology.
97. Minagar, A. & Alexander, J. S. Blood–brain barrier disruption in multiple sclerosis. *Mult. Scler.* **9**, 540–549 (2003).
98. Abbott, N. J. *et al.* in *Mechanisms of Drug Resistance in Epilepsy: Lessons from Oncology* (ed. Ling, V.) Novartis Foundation Symposium No. 243, 38–47 (John Wiley, Chichester, UK, 2002).
99. Marroni, M. *et al.* Vascular and parenchymal mechanisms in multiple drug resistance: a lesson from human epilepsy. *Curr. Drug Targets* **4**, 297–304 (2003).
100. Zlokovic, B. V. Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci.* **28**, 202–208 (2005).
101. Kortekaas, R. *et al.* Blood–brain barrier dysfunction in parkinsonian midbrain *in vivo*. *Ann. Neurol.* **57**, 176–179 (2005).
An important but controversial paper showing how modern imaging techniques can be used to investigate BBB transport function in humans, and the insight this may give into disease states.
102. Schwaninger, M. *et al.* Bradykinin induces interleukin-6 expression in astrocytes through activation of nuclear factor-κB. *J. Neurochem.* **73**, 1461–1466 (1999).
103. Deli, M. A. *et al.* Exposure of tumor necrosis factor-α to luminal membrane of bovine capillary endothelial cells cocultured with astrocytes induces a delayed increase of permeability and cytoplasmic stress formation of actine. *J. Neurosci. Res.* **41**, 717–726 (1995).
104. Didier, N. *et al.* Secretion of interleukin-1β by astrocytes mediates endothelin-1 and tumour necrosis factor-α effects on human brain microvascular endothelial cell permeability. *J. Neurochem.* **86**, 246–254 (2003).
Illustrates the potential complexities of signalling between cells at the BBB — even apparently direct actions may involve indirect loops and potentiating (and inhibitory) modulation.
105. Perry, V. H., Newman, T. A. & Cunningham, C. The impact of systemic infection on the progression of neurodegenerative disease. *Nature Rev. Neurosci*. **4**, 103–112 (2003).
106. Banks, W. A. Blood–brain barrier transport of cytokines: a mechanism for neuropathology. *Curr. Pharm. Des.* **11**, 973–984 (2005).
107. Watkins, L. R. & Maier, S. F. Glia: a novel drug discovery target for clinical pain. *Nature Rev. Drug Discov.* **2**, 973–985 (2003).
108. Huber, J. D. *et al.* Inflammatory pain alters blood–brain barrier permeability and tight junctional protein expression. *Am. J. Physiol. Heart Circ. Physiol*. **280**, H1241–H1248 (2001).
Recent work has shown, rather surprisingly, that even peripheral stimuli such as inflammatory pain can open the BBB.
109. Abbott, N. J. Prediction of blood–brain barrier permeation in drug discovery, from *in vivo*, *in vitro* and *in silico* models. *Drug Discov. Today: Technologies* **1**, 407–416 (2004).
110. Dietrich, J. B. Endothelial cells of the blood–brain barrier: a target for glucocorticoids and estrogens? *Front. Biosci.* **9**, 684–693 (2004).
111. Krizanac-Bengez, L., Mayberg, M. R. & Janigro, D. The cerebral vasculature as a therapeutic target for neurological disorders and the role of shear stress in vascular homeostasis and pathophysiology. *Neurol. Res.* **26**, 846–853 (2004).
112. Demeule, M. *et al.* Brain endothelial cells as pharmacological targets in brain tumors. *Mol. Neurobiol.* **30**, 157–183 (2004).
113. Kaal, E. C. & Vecht, C. J. The management of brain edema in brain tumors. *Curr. Opin. Oncol.* **16**, 593–600 (2004).
114. Cucullo, L., Hallene, K., Dini, G., Dal Toso, R. & Janigro, D. Glycerophosphoinositol and dexamethasone improve transendothelial electrical resistance in an *in vitro* study of the blood–brain barrier. *Brain Res.* **997**, 147–151 (2004).
115. Brown, R. C., Mark, K. S., Egleton, R. D. & Davis, T. P. Protection against hypoxia-induced blood–brain barrier disruption: changes in intracellular calcium. *Am. J. Cell Physiol.* **286**, C1045–C1052 (2004).
116. Turkel, N. A. & Ziylan, Z. Y. Protection of blood–brain barrier breakdown by nifedipine in adrenaline-induced hypertension. *Int. J. Neurosci.* **114**, 517–528 (2004).
117. Preston, E. & Webster, J. A two-hour window for hypothermic modulation of early events that impact delayed opening of the rat blood–brain barrier after ischemia. *Acta Neuropathol. (Berl.)* **108**, 406–412 (2004).
118. Wagner, K. R. & Zuccarello, M. Local brain hypothermia for neuroprotection in stroke treatment and aneurysm repair. *Neurol. Res.* **27**, 238–245 (2005).
119. Park, S. *et al.* Neurovascular protection reduces early brain injury after subarachnoid hemorrhage. *Stroke* **35**, 2412–2417 (2004).
120. Franzén, B. *et al.* Gene and protein expression profiling of human cerebral endothelial cells activated with tumor necrosis factor-α. *Mol. Brain Res.* **115**, 130–146 (2003).
With the human genome now fully sequenced, efforts are being made to identify genes and proteins of the brain endothelium that are activated in inflammation and disease, and that could therefore be useful targets for therapy and drug delivery to the brain. This is one of the first reports.
121. Kaya, D. *et al.* VEGF protects brain against focal ischemia without increasing blood–brain barrier permeability when administered intracerebro-ventricularly. *J. Cereb. Blood Flow Metab*. **25**, 1111–1118 (2005).
122. Takahashi, M. & Macdonald. R. L. Vascular aspects of neuroprotection. *Neurol. Res.* **26**, 862–869 (2004).
123. Rapoport, S. I. Advances in osmotic opening of the blood–brain barrier to enhance CNS chemotherapy. *Expert Opin. Invest. Drugs* **10**, 1809–1818 (2001).
124. Kraemer, D. F., Fortin, D. & Neuwelt, E. A. Chemotherapeutic dose intensification for treatment of malignant brain tumors: recent developments and future directions. *Curr. Neurol. Neurosci. Rep*. **2**, 216–224 (2002).
125. Farkas, A. *et al.* Hyperosmotic mannitol induces Src kinase-dependent phosphorylation of β-catenin in cerebral endothelial cells. *J. Neurosci. Res*. **80**, 855–861 (2005).
126. Prados, M. D. *et al.* A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma. *Neuro-oncology* **5**, 96–103 (2003).
127. Ashraf, M. Z., Hussain, M. E. & Fahim, M. Antiatherosclerotic effects of dietary supplementations of garlic and turmeric: restoration of endothelial function in rats. *Life. Sci.* **77**, 837–857 (2005).
128. Rohdewald, P. A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology. *Int. J. Clin. Pharmacol. Ther.* **40**, 158–168 (2002).

129. Bijl, M. Endothelial activation, endothelial dysfunction, and premature atherosclerosis in systemic autoimmune diseases. *Neth. J. Med.* **61**, 273–277 (2003).
130. Calabresi, L., Gomaraschi, M. & Franceschini, G. Endothelial protection by high-density lipoproteins. From bench to bedside. *Arterioscler. Thromb. Vasc. Biol.* **23**, 1724–1731 (2003).
131. d'Alessio, P. Aging and the endothelium. *Exp. Gerontol.* **39**, 165–171 (2004).
132. Middlebrook, A. R. *et al.* Does aerobic fitness influence microvascular function in healthy adults at risk of developing type 2 diabetes? *Diabet. Med.* **22**, 483–489 (2005).
133. Abeywardena, M. Y. & Head, R. J. Longchain *n*-3 polyunsaturated fatty acids and blood vessel function. *Cardiovasc. Res.* **52**, 361–371 (2001).
134. De Caterina, R., Madonna, R. & Massaro, M. Effects of omega-3 fatty acids on cytokines and adhesion molecules. *Curr. Atheroscler. Rep.* **6**, 485–491 (2004).
135. Harris, H. W., Rockey, D. C., Young, D. M. & Welch, W. J. Diet-induced protection against lipopolysaccharide includes increased hepatic NO production. *J. Surg. Res.* **82**, 339–345 (1999).
136. Kamata, K. *et al.* Effects of chronic administration of fruit extract (*Citrus unshiu* Marc) on endothelial dysfunction in streptozotocin-induced diabetic rats. *Biol. Pharm. Bull.* **28**, 267–270 (2005).
137. Hwang, J., Hodis, H. N. & Sevanian, A. Soy and alfafa phytoestrogen extracts become potent low-density lipoprotein antioxidants in the presence of acerola cherry extract. *J. Agric. Food Chem.* **49**, 308–314 (2001).
138. Vera, R. *et al.* Soy isoflavones improve endothelial function in spontaneously hypertensive rats in an estrogen-independent manner: role of nitric-oxide synthase, superoxide, and cyclooxygenase metabolites. *J. Pharmacol. Exp. Ther.* **314**, 1300–1309 (2005).
139. d'Uscio, L. V., Milstein, S., Richardson, D., Smith, L. & Katusic, Z. S. Long-term vitamin C treatment increases vascular tetrahydrobiopterin levels and nitric oxide synthase activity. *Circ. Res.* **92**, 88–95 (2003).
140. Marsh, S. A., Laursen, P. B., Pat, B. K., Gobe, G. C. & Coombes, J. J. Bcl-2 in endothelial cells is increased by vitamin E and α-lipoic acid supplementation but not exercise training. *J. Mol. Cell. Cardiol.* **38**, 445–451 (2005).
141. Praticò, D. Antioxidants and endothelium protection. *Atherosclerosis* **181**, 215–224 (2005).
142. Rasmussen, S. E., Frederiksen, H., Struntze Krogholm, K. & Poulsen, L. Dietary proanthocyanidins: occurrence, dietary intake, bioavailability, and protection aganist cardiovascular disease. *Mol. Nutr. Food Res.* **49**, 159–174 (2005).
143. Fung, T. T. *et al.* Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. *Am. J. Clin. Nutr.* **82**, 163–173 (2005).
This study on the effect of diet on systemic endothelial function is a useful indicator of possible ways to maintain a healthy BBB.
144. Youdim, K. A., Spencer, J. P., Schroeter, H. & Rice-Evans, C. Dietary flavonoids as potential neuroprotectants. *Biol. Chem.* **383**, 503–519 (2002).
145. Yoshida, H. *et al.* Inhibitory effect of tea flavonoids on the ability of cells to oxidize low density lipoprotein. *Biochem. Pharmacol.* **58**, 1695–1703 (1999).
146. Stoclet, J. C. *et al.* Vascular protection by dietary polyphenols. *Eur. J. Pharmacol.* **500**, 299–313 (2004).
147. Kawakami, M., Sekiguchi, M., Sato, K., Kozaki, S. & Takahashi, M. Erythropoietin receptor-mediated inhibition of exocytotic glutamate release confers neuroprotection during chemical ischemia. *J. Biol. Chem.* **276**, 39469–39475 (2001).
148. Martínez-Estrada, O. M. *et al.* Erythropoietin protects the *in vitro* blood–brain barrier against VEGF-induced permeability. *Eur. J. Neurosci.* **18**, 2538–2544 (2003).
149. Zonta, M. *et al.* Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nature Neurosci.* **6**, 43–50 (2003).
150. Tran, N. D., Correale, J., Schrieber, S. S. & Fisher, M. Transforming growth factor-β mediates astrocyte-specific regulation of brain endothelial anticoagulant factors. *Stroke* **30**, 1671–1677 (1999).
151. Lo, E. H., Dalkara, T. & Moskowitz, M. A. Mechanisms, challenges and opportunities in stroke. *Nature Rev. Neurosci.* **4**, 399–415 (2003).
152. Tomas-Camardiel, M. *et al.* Blood–brain barrier disruption highly induces aquaporin-4 mRNA and protein in perivascular and parenchymal astrocytes: protective effect by estradiol treatment in ovariectomized animals. *J. Neurosci. Res.* **80**, 235–246 (2005).
153. Vakili, A., Kataoka, H. & Plesnila, N. Role of arginine vasopressin V_1 and V_2 receptors for brain damage after transient focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* **25**, 1012–1019 (2005).
154. Gaillard, P. J., de Boer, A. B. & Breimer, D. D. Pharmacological investigations on lipopolysaccharide-induced permeability changes in the blood–brain barrier *in vitro*. *Microvasc. Res.* **65**, 24–31 (2003).
155. Veldhuis, W. B. *et al.* Interferon-β prevents cytokine-induced neutrophil infiltration and attenuates blood–brain barrier disruption. *J. Cereb. Blood Flow Metab.* **23**, 1060–1069 (2003).
156. Oki, T. *et al.* Increased ability of peripheral blood lymphocytes to degrade laminin in multiple sclerosis. *J. Neurol. Sci.* **222**, 7–11 (2004).
157. Dallasta, L. M. *et al.* Blood–brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. *Am. J. Pathol.* **155**, 1915–1927 (1999).
158. Berger, J. R. & Avison, M. The blood brain barrier in HIV infection. *Front. Biosci.* **9**, 2680–2685 (2004).
159. Kalaria, R. N. The blood–brain barrier and cerebrovascular pathology in Alzheimer's disease. *Ann. NY Acad. Sci.* **893**, 113–125 (1999).
160. Lee, G. & Bendayan, R. Functional expression and localization of P-glycoprotein in the central nervous system: relevance to the pathogenesis and treatment of neurological disorders. *Pharm. Res.* **21**, 1313–1320 (2004).
161. Papadopoulos, M. C., Saadoun, S., Davies, D. C. & Bell, B. A. Emerging molecular mechanisms of brain tumour oedema. *Br. J. Neurosurg.* **15**, 101–108 (2001).
162. Davies, D. C. Blood–brain barrier breakdown in septic encephalopathy and brain tumours. *J. Anat.* **200**, 639–646 (2002).
163. Segal, M. B. & Zlokovic, B. V. *The Blood–Brain Barrier, Amino Acids and Peptides* (Kluwer Academic, Dordrecht, Boston (USA) & London (UK), 1990).

Acknowledgements
The work performed in the authors' laboratories was supported by grants from the Wellcome Trust (N.J.A.) and the Swedish Research Council (L.R. and E.H.). Figures 2 and 6 were originally designed by E. Kraft, Göteborg, Sweden, who is gratefully acknowledged.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ANG1 | AQP4 | CASK | claudin 3 | claudin 5 | claudin 12 | EAAT1–3 | GLUT1 | JACOP | JAM-A | JAM-B | JAM-C | LAT1 | MAGI-1 | MAGI-2 | MAGI-3 | MUPP1 | occludin | PAR3 | PAR6 | PXR | RGS5 | TIE2 | ZO-1 | ZO-2 | ZO-3 | ZONAB

FURTHER INFORMATION
Abbott's homepage: http://www.kcl.ac.uk/depsta/biomedical/CARD/abbott_profile.htm
Access to this interactive links box is free online.