FEBS Letters 588 (2014) 1259–1270

FEBS Letters
journal homepage: www.FEBSLetters.org

Review

A new angle on blood–CNS interfaces: A role for connexins?

Marijke De Bock [a,1], Roosmarijn E. Vandenbroucke [b,c,1], Elke Decrock [a], Maxime Culot [d], Romeo Cecchelli [d], Luc Leybaert [a,*]

[a] Department of Basic Medical Sciences, Physiology Group, Ghent University, Ghent, Belgium
[b] Inflammation Research Center, VIB, Ghent, Belgium
[c] Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
[d] Laboratoire de Physiopathologie de la Barrière Hémato-Encéphalique, Faculté Jean Perrin, Université Lille Nord de France, Lens, France

ARTICLE INFO

Article history:
Received 5 February 2014
Revised 27 February 2014
Accepted 28 February 2014
Available online 12 March 2014

Edited by Michael Koval, Brant E. Isakson, Robert G. Gourdie and Wilhelm Just

Keywords:
Connexin
Hemichannel
Gap junctions
Blood–brain barrier
Choroid plexus epithelium
Junctional complex

ABSTRACT

Neuronal signaling in the CNS depends on the microenvironment around synapses and axons. To prevent fluctuations in blood composition affecting the interstitial fluid and CSF, two barriers, the blood–brain barrier (BBB) and blood–CSF barrier (BCSFB), are interposed between the blood and the brain/CSF compartment. Brain capillary endothelial cells (ECs) constitute the BBB whereas choroid plexus epithelial (CPE) cells form the BCSFB. The anatomical basis of these barriers is located at the level of an intercellular junctional complex that impedes paracellular diffusion. Tight and adherens junctions are known as the principal constituents of this junctional complex. Transmembrane connexins (Cxs) are the prime building blocks of plasma membrane hemichannels that combine to form intercellular gap junctions (GJ). Although Cxs co-exist within the junctional complex, their influence on tight/adherens junctions and their role in barrier function of BBB ECs and CPE has been mostly ignored. Here, we review current knowledge on the role of Cxs in the BBB, BCSFB and other interfaces that subside within the CNS. We conclude that Cxs are a rather unexplored but promising target for influencing CNS barrier function.

1. Barriers in the CNS

Efficient electrical signaling in the CNS requires a balanced and well-controlled composition of the micro-environment around glial cells, neurons and synapses. This is maintained by a series of barriers interposed between the nervous tissue, the blood and the cerebrospinal fluid (CSF) that collectively protect the interstitial fluid and CSF from fluctuations in blood composition. The largest, most stringent, and by far, most complex barrier is formed by capillary endothelial cells (ECs) and is situated between the blood and the brain (i.e., blood–brain barrier, BBB), whereas epithelial cells of the choroid plexus, as well as the arachnoid epithelium and tanycytes form a blood–CSF barrier (BCSFB). Tight junctions (TJs) and adherens junctions (AJs) between the endothelial and epithelial cells are the structural components of these barriers as they impede the paracellular movement of ions, solutes, proteins, water and blood cells. This implies the necessity for transporter mechanisms that mediate the influx of essential, and the efflux of deleterious molecules across the barriers.

The expression and function of TJs and AJs in the barriers of the CNS is well established; however, our appreciation of expression and function of a third class of junctional proteins, i.e. connexins, is lagging far behind. In this review we summarize current knowledge on the expression and role of these proteins at the different CNS interfaces.

1.1. The blood–brain barrier

The distinct and unique capillary endothelial cells (ECs) of the brain form the anatomical basis of the vertebrate blood–brain barrier (BBB) (Fig. 1). These cells furnish a highly sophisticated junctional complex consisting of AJs and TJs that limit paracellular diffusion of solutes between the blood and the brain [1–4]. This tempered paracellular movement of water, molecules and ions is echoed in a high transendothelial electrical resistance (TEER). Electrical resistance measured in frog pial capillaries amounts 1900 $\Omega.cm^2$ and values measured in rat cerebral capillaries are in the range of 3000 $\Omega.cm^2$ [5]. In comparison, peripheral microvessels, that are often fenestrated and exhibit slightly different TJ components and

* Corresponding author. Address: Department of Basic Medical Sciences, Physiology Group, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, Building B (Rm 310), 9000 Ghent, Belgium. Fax: +32 9 332 30 59.
E-mail address: Luc.Leybaert@UGent.be (L. Leybaert).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.febslet.2014.02.060
0014-5793/

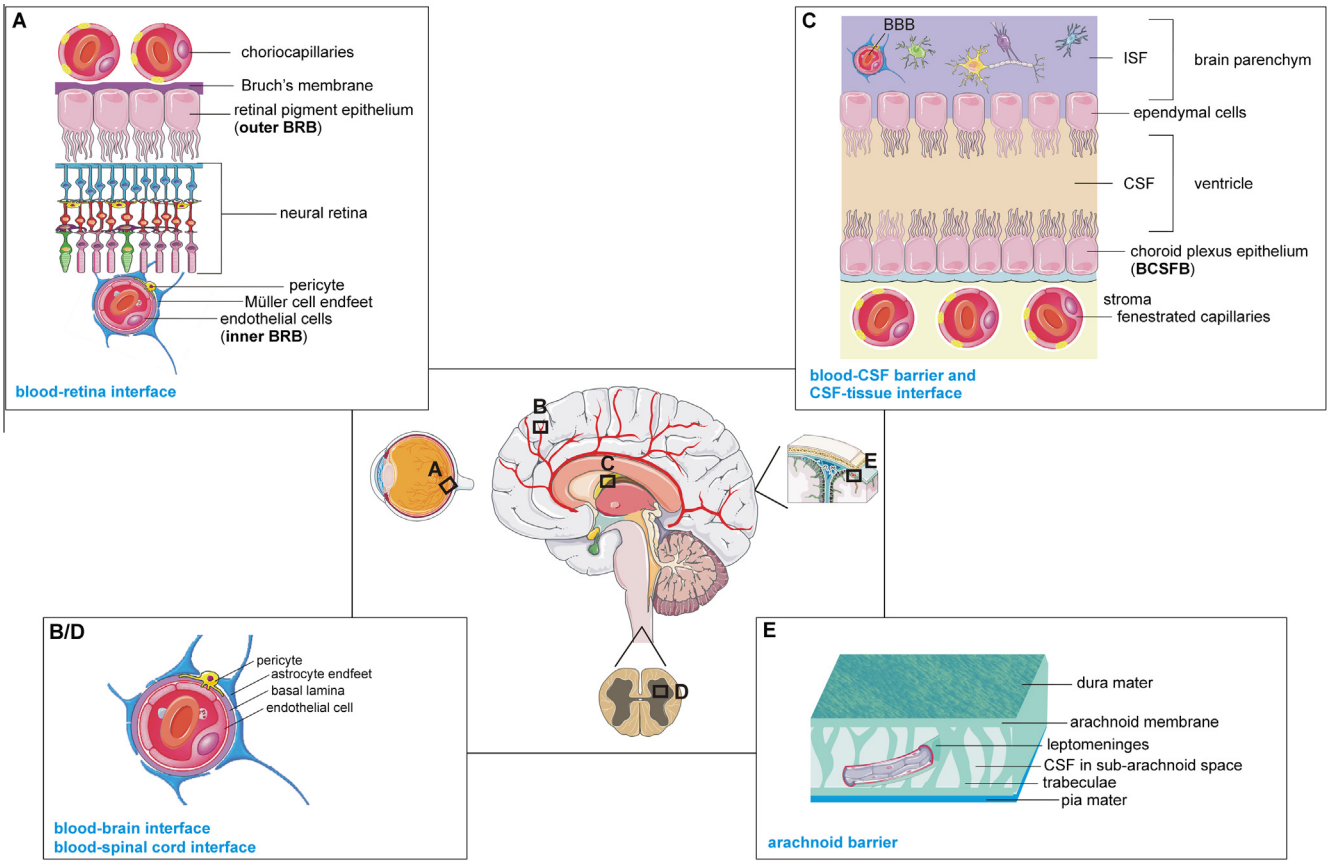

Fig. 1. Barriers of the CNS. The central nervous system, i.e. brain, spinal cord and retina, is separated from the peripheral blood by a set of epithelial and endothelial barriers. The central image depicts a schematic representation of the CNS with insets pointing to the location of the different blood–tissue interfaces. The detailed architecture of the separate barriers is illustrated in the outer panels. At the ocular level (panel A), the inner endothelial and outer epithelial BRB act together to protect the retina from potentially deleterious circulating molecules. In the brain (panel B/D), the endothelial BBB is interposed between the nervous tissue and the microcirculation, while at the ventricular level (panel C), the epithelial BCSFB forms a blood–CSF interface. In the spinal cord, the BSCB, an endothelial barrier highly similar to that in the brain (panel B/D), can be found. The arachnoid barrier which is composed of leptomeningeal cells finally separates the blood compartment from the CSF in the sub-arachnoid space (panel E).

organization [6,7] have TEER values a 100 times lower. The absence of pinocytotic activity and the presence of a strictly regulated set of transport proteins and enzymes add up to the specific features of BBB ECs [1], altogether rendering these vessels far less permeable to endo- and exogenous molecules than their peripheral counterparts. These specialized characteristics may endure in pre- and post-capillary segments, though it is not clear to what extent [1–3].

TJs or *zonulae occludens* (ZO) are domains of occluded intercellular clefts that in freeze-fracture replicas appear as an elaborate complex of parallel, anastomosing intramembranous protein strands, arranged as a series of multiple barriers (Fig. 2). They not only restrict the paracellular passage of solutes (gate function), but also polarize the cell (fence function) [8,9]. Claudins are a family of transmembrane (TM) proteins (20–24 kDa) of which more than 24 isoforms are identified [10] and are the primary barrier-forming TJ constituents. In the BBB, claudin-3, -5 and -12 are most important for composing the TJ backbone [4,11,12], but claudin-2, -11, and -18 have been identified as well [13]. Occludin was the first TJ protein discovered [14] but in contrast to claudins, it does not seem to be required for the formation of TJs as such. Well-developed, morphologically correct networks of TJ strands have indeed been observed in occludin deficient animals [15]. Furthermore, occludin cannot establish organized strands by itself but is instead incorporated into claudin-based strands [16]. Nevertheless, functionally, occludin expression is associated with TJ sealing and is correlated with increased electrical resistance [17,18]. In this respect, strong occludin expression is mainly observed in BBB endothelium and other CNS barriers (see further) whereas only trace amounts are observed in ECs of peripheral tissue like the aorta [19,20]. Mature BBB ECs thus use occludin to regulate rather than to establish their barrier properties. The JAM proteins represent a third family of TM proteins that mediate homo- and heterophilic interactions in the TJ region, establishing the early attachment of adjacent cell membranes [21,22]. Other, less-characterized TM proteins, like junctional adhesion molecules (JAMs) and tricellulin, may further assist in TJ formation [23]. First- and second-order cytoplasmic adaptor proteins are associated with the TM components and act as scaffolds for cytoskeletal and signaling molecules. ZO proteins are the prototypic example of such first-order adaptor proteins. They play an important role in signal transduction and in anchoring the TJ proteins to the cytoskeleton, either *via* direct interaction with actin or indirectly *via* binding to second-order adaptor proteins. This association is not only important for stabilization of the junctions, but also for the dynamic regulation of junction opening and closure [6,23]. Brain microvessels are especially abundant in ZO-2 with a contribution of ZO-1, but ZO-3 is hardly detected [19]. Other cytoplasmic adaptor proteins include cingulin, afadin (AF-6) and 7H6 antigen [24].

Already during fetal development, TJs are present between ECs of the vascular sprouts that invade the neural tube. Although these early vessels display an enlarged diameter and irregular shape, the absence of plasma proteins in the brain interstitial compartment and the impermeability of 3 kDa biotin-dextrans suggest that paracellular diffusion is restricted and that TJs are sufficiently well

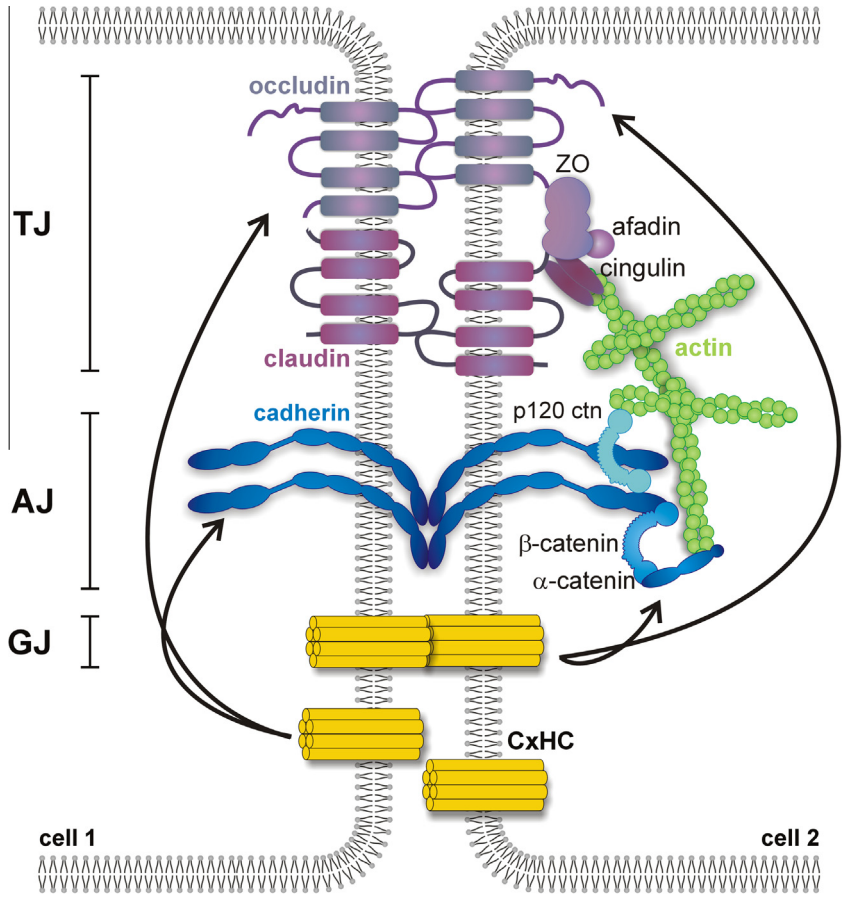

Fig. 2. Cx channels form part of the intercellular junctional complex. TJs and AJs between endothelial and epithelial cells are responsible for impeding the paracellular movement of ions, solutes and blood cells; thereby establishing the anatomical barriers of the CNS. The principal constituents of TJs are the transmembrane proteins occludin and claudins. These interact with the actin cytoskeleton via adaptor proteins such as the ZO proteins, afadin and cingulin. AJs are build up by transmembrane proteins belonging to the cadherin family and by catenins that link the cadherins to actin. Transmembrane Cx proteins constitute a third partner in the intercellular junctional complex. These proteins combine into a CxHC that exist as transmembrane channels in the plasma membrane or that exist as GJ channels, formed by two apposed CxHCs. GJ channels are found within the junctional complex, are endowed with adhesive properties and functionally link the cytoplasm of adjacent cells. Cx channels may connect physically to claudins, occludin, N-cadherin and catenins, or may promote occlusion of the intercellular cleft by indirect signaling to AJs and TJs (indicated by the arrows).

developed to exclude these proteins and markers from the fetal brain parenchyma [8,25]. *In situ* measurement of electrical resistance over the pial vessel wall reported a low TEER (~300 $\Omega.cm^2$) in rat embryos up to E20, but starting from E21, TEER increases up to ~1200 $\Omega.cm^2$ [26]. The further maturation of the barrier arises from progressive changes in TJ morphology which is initiated by interactions between ECs and their surrounding partners [8,25,27,28].

The current concept of the BBB has shifted from a purely anatomical, endothelial barrier to an integrative view in which the BBB is regarded a modulatory interface, controlled by intercellular signaling processes between ECs, glial cells, pericytes and neurons that together form the neurogliovascular unit (or neurovascular unit) [1,29]. Astrocytic projections, termed endfeet, almost completely surround the brain capillaries, but direct contact between these structures and the endothelium is hindered by the basement membrane [30]. Grafting experiments are indicative for the barrier-supporting role of the nervous tissue: brain vessels growing into peripheral tissue grafts become less tight, whereas leaky vessels become more restrictive when grafted into brain tissue [31]. However, although astrocytes are necessary for BBB maintenance, they are probably not sufficient to induce a proper barrier. Some BBB characteristics are present very early during development, even before differentiated astrocytes are present. In rodents, neurogenesis (E11–17), rather than gliogenesis (E17) coincides with the invasion of early blood vessels into the neural tube (E10–11) [25,27]. Therefore, neurons or pericytes are more likely candidates to induce TJ formation while gliogenesis coincides with marked alterations in TJ complexity [32]. Pericytes are in close contact with capillaries, even in the earliest stages of development. They display a dendrite morphology with several cytoplasmic processes wrapping around the vessel wall [33] and are, together with the ECs, embedded in the endothelial basement membrane of which they synthetize most molecular components [13,34]. Unlike astrocytes, pericytes are in direct contact with ECs. Pericytes regulate vessel growth and development, as well as TJ formation and transporter function *via* secretion of growth factors such as transforming growth factor-β (TGFβ) angiopoietin-1, platelet-derived growth factor B (PDGF-B) and basic fibroblast growth factor (bFGF) [34–41]. In higher order vessels, these growth factors are additionally involved in regulation of vessel tone [42–44].

1.2. The blood–CSF barriers

The brain is surrounded, both internally and externally, by CSF which is secreted by the choroid plexus, a villous structure located in the roof of each of the four cerebral ventricles. The choroid plexus is a highly vascularized structure; yet capillaires are fenestrated and leaky, thus lacking barrier properties. Nevertheless, a barrier between blood and CSF is formed by the cuboidal choroid plexus epithelial cells (CPE) that are positioned between these

two compartments (Fig. 1). The TJ structure at the BBB and the BCSFB is largely similar, but ECs and CPE express different levels of occludin, ZO-1 and ZO-2 [19]. In addition, different claudin isoforms are present in these cells. As described above, BBB ECs mainly express claudins-3, -5 and -12 whereas claudins-1, -2, -3 and -11 are found in the CPE [19,45,46]. These varying isoforms and expression levels may account for the differences in resistance measured over the BBB and BCSFB. It is challenging to measure electrical resistance over the BCSFB *in vivo*; yet *in vitro* measurements of resistance over CPE isolated from bull frog is about 150 $\Omega.cm^2$ [47], much lower compared to that measured at the level of the BBB. While the BBB is considered an absolute immunological barrier, the BCSFB is thought to be permissive to immune surveillance of the CNS [48], further highlighting important differences between the BBB and the BCSFB. Major functions of the CPE include the secretion of CSF for the ventricles and the production of growth factors *via* which they signal to the fenestrated capillaries [49]. The CPE is highly responsive to inflammatory changes in the periphery, which is reflected in inflammation-induced changes in CPE proteome, secretome, transcriptome and functionality [50–52]. The outer layer of the ventricular lining is composed of ependymal cells that constitute a leaky CSF–brain interface. Indeed, despite the presence of occludin, ZO-1 and AJ proteins, the TJs in ependymal cells are discontinuous, blind-ending strands [53,54]. As a result, the CSF is in contact with and mixes with the brain interstitial fluid which has a similar composition [55] (Fig. 1). Nevertheless, ependymal cells express a multitude of enzymes and transporters *via* which they regulate the exchange between CSF and interstitial fluid. Glucose levels for example differ between the two compartments (CSF: ~2.5 mM *versus* brain interstitial fluid: ~1 mM) [56,57]. Ependymal cells additionally scavenge toxic byproducts of metabolism thereby constituting a metabolic barrier. Also, by regulating water flow through aquaporin channels, ependymal cells are important for handling hydrocephalic pressure [53].

A second blood–CSF interface is located at the level of the circumventricular organs (CVOs), such as the median eminence (ME), i.e. the neurohemal part of the hypothalamus where hormones are collected before entering the hypophyseal portal system. CSF communicates with the internal milieu of the ME but a barrier is present between the arcuate nucleus, where hormones are secreted, and the ME, preventing blood coming into contact with the CSF. This barrier is formed by tanycytes that are found predominantly lining the floor of the 3rd ventricle, overlying the ME [58] and that face the fenestrated portal capillaries of the ME, forming a dense network around them. TJ proteins were identified as a continuous belt around the tanycyte cell bodies and to form a diffusion barrier for the large MW tracer Evans Blue [59]. Recently, a similar barrier was observed in other CVOs including the *organum vasculosum laminae terminals* (OVLT), the *subfornical organ* (SFO) and the *area postrema* (AP) [60]. The characterization of TJ components in tanycytes is less straightforward. Tanycytes describe a heterogeneous population of $alpha_{1/2}$ and $beta_{1/2}$ subtypes that are all adjoined by AJs, but although TJ proteins are present in $alpha_2$, $beta_1$ and $beta_2$ tanycytes, actual TJs were only identified between beta cells. Hence, only $beta_1$ and $beta_2$ tanycytes have been proposed to form a diffusion barrier [61].

Finally, at the exterior part of the brain, three layers of meninges, namely the *dura mater*, arachnoid membrane and *pia mater* cover the brain surface. Fenestrated vessels are found throughout the meninges, but due to the presence of TJs in the outer arachnoid leptomeningeal membrane, blood does not come into contact with the subarachnoid CSF that is drained from the ventricular space [45,46,62–64].

In both the BBB and BCSFB, simple paracellular diffusion of metabolites and ions is impeded by the presence of TJs in endothelial and epithelial cells respectively. Consequently, exchange between blood and nervous tissue is largely dependent on solute carriers that provide essential nutrients to the brain. These include different amino acid transporters, GLUT-1 (glucose uptake) and ion transporters. Extrusion of toxic waste products largely depends on a variety of energy-requiring efflux pumps (ABC transporters) that remove deleterious lipophilic compounds diffusing in over the BBB. P-glycoprotein is without doubt the best known example of such efflux pump. The BBB and the BCSFB have, in general, a similar functional organization with regard to transport of molecules, but the actual set of carrier proteins and efflux pumps slightly differs. An elaborate description of transporters in the blood–brain and blood–CSF interfaces lies beyond the scope of this review but is excellently reviewed in [46,65].

1.3. Other barriers in the CNS

The main supply of blood to the retina, that is ontogenetically part of the CNS, is provided by retinal microvessels whereas the outer one-third is perfused by the choroidal circulation. At both levels, a blood–retinal barrier (BRB) separates blood from the retinal nervous tissue (Fig. 1). The inner BRB (iBRB) is highly similar to the BBB and is composed of capillary, non-fenestrated ECs that are characterized by highly restrictive TJs. These ECs lie on a basement membrane that also contains pericytes, and that is associated with astrocytic endfeet and glial Muller cells. The outer BRB (oBRB) is located at the retinal pigment epithelium (RPE) that lies on the Bruch's membrane and that contains TJs separating the fenestrated choriocapillaires from the neural retina. Like the BBB and BCSFB, the oBRB and iBRB jointly serve to secure a continuous nutrient supply and metabolite removal in order to maintain a stable composition of the extracellular fluid in the retina [66,67].

Finally, an endothelial barrier is present between the blood and the spinal cord; the BSCB. This barrier is roughly similar to the BBB and both are often mentioned in the same breath. More and more evidence indicates however, that important structural and functional differences exist between the BBB and the BSCB. The BSCB for instance contains lower levels of occludin, ZO-1 and AJ proteins VE-cadherin and β-catenin. As a result, the barrier is more permeable to tracers and pro-inflammatory cytokines. Additionally, BSCB ECs express lower levels of P-glycoprotein, the major efflux pump in the BBB [68]. Further studies will be required to define and characterize additional differences between the BBB and the BSCB. It is not clear whether a BCSFB exists in the spinal cord, where CSF flows in the central canal and in the subarachnoid space that is continuous with the subarachnoid space around the brain.

2. Connexins, not just another partner in the junctional complex

Although often overlooked when discussing intercellular junctions, connexin (Cx) proteins constitute an important partner in the junctional complex [69,70] (Fig. 2). Cxs belong to a superfamily containing 21 isoforms that are expressed in a tissue-specific manner and which are named according to their molecular weight (e.g. Cx43 has a MW of 43 kDa) [71,72]. The Cxs appear in a hexameric configuration in the plasma membrane (PM), but unlike TJs and AJs proteins, these do not form a tight seal between the cells. Their primary function is to form gap junction (GJ) channels that directly connect the cytoplasm of adjacent cells. GJs are also endowed with adhesive properties, for example to attach neurons to radial glial cells during their cortical migration in brain development [73–77].

GJ channels are large, poorly selective, aqueous pores that are assembled by the head-to-head docking of two half GJ channels or hemi-channels (CxHC), each delivered by one of the partner cells. CxHCs are composed of six Cx proteins, arranged around a central pore. CxHC docking occurs in specialized PM regions termed GJ plaques which can be found in nearly all mammalian cell types [78–80]. GJ plaques are characterized in freeze-fracture microscopy by clusters of condense membrane particles with central depressions corresponding to the channel pores. The plaques contain from less than a dozen to up to 200000 units and may extend from several nanometers to a few micrometers in diameter [78,79]. CxHCs, by themselves, form a tightly regulated conduit between the cytoplasm and the extracellular environment. Because CxHCs allow the passage of globular substances up to ~1.5 kDa, it was originally believed that these channels remain closed until they dock with opposed CxHCs to form GJ channels. This would prevent cell death caused by the loss of essential metabolites, energy substrates and diffusible second messengers, and by the collapse of ionic gradients. However, brief and controlled opening of CxHCs does not lead to cell loss, suggesting that cells can at least cope with some degree of CxHC opening [81]. CxHCs may be activated by a multitude of extra- and intracellular triggers such as changes in the intra- ($[Ca^{2+}]_i$) and extracellular ($[Ca^{2+}]_e$) Ca^{2+} concentration, free radicals, intracellular redox potential [82–85], metabolic inhibition [86] or ischemia [87], and mechanical stimulation or fluid flow shear stress [88]. Whereas GJs form a direct communication route between cells, CxHC opening provides a diffusive uptake and release pathway. CxHCs have been demonstrated to allow the release of messenger molecules like ATP and glutamate [89], thereby possibly contributing to both autocrine and paracrine signaling [90]. Together, GJs and CxHCs coordinate different functions from the cellular up to the organ level, including development and differentiation, oncogenic transformation and growth control, cell death, inflammation and intercellular signal transmission [91–93].

Cxs significantly contribute to the junctional complex. Recent research has revealed the involvement of Cxs in stabilizing intercellular junctions, thereby promoting occlusion of the intercellular cleft in a number of peripheral tissues [94]. Several lines of evidence confirm this notion. Freeze-fracture analysis has revealed the presence of GJs plaques within TJ strand networks [67,95,96]. In colonic epithelial cells, airway epithelial cells and hepatocytes, Cxs potentiate the expression of and interact with claudins, occludin, N-cadherin, and catenins [69,95,97–102]. These interactions are suggested to enhance barrier function. Oppositely, treatment with Cx channel blockers downregulates the expression of occludin and claudins, increasing the permeability of the different compartmental barriers [95,102–105]. The role of Cxs in modulating barrier function is most elegantly demonstrated in the testis, where a barrier is formed by the seminiferous Sertoli cells. Here, Cx43 is transiently lost from the intercellular junctions during the spermiation process which involves a temporary increase in permeability. Cx43 re-associates with the junctional proteins as soon as the barrier closes up again [106].

From the data discussed above, it follows that Cxs provide an important contribution to the barrier function established by TJs and AJs in peripheral tissues; however, Cxs are often neglected in the context of CNS barriers. In the following chapter we will provide evidence that Cxs are also important players in regulating the permeability of the blood–CSF and blood–tissue interfaces.

3. Connexins in the cerebral barriers

Cxs are found throughout the CNS where they establish connections that are most prominent in glial and vascular cells, but also exist in neurons. Eleven Cx subtypes are found in the CNS with the subset and the expression levels varying depending on the cell type and the developmental stage [107]. GJs and CxHCs are involved in key functions of the CNS such as glial metabolism and synaptic signaling, and aberrant Cx protein expression may contribute to CNS pathologies (reviewed in [92,107–110]). However, the role of Cxs in the barrier-forming cells of the CNS has hardly received attention.

3.1. Cxs in endothelial cells of the BBB, BRB and BSCB

Vascular ECs throughout the body express Cx37, Cx40 and Cx43 with variability in abundance, depending on the vessel type and position in the vascular tree. Cx37 and Cx40 are widely distributed in large vessel ECs, whereas Cx43 is strongly expressed in regions of turbulent flow [111–113]. Our knowledge on the role of Cx channels in the function of capillaries and microvessels is still limited. In larger vessels (arteries/veins and arterioles/venules) GJs coordinate cell migration during angiogenesis and wound healing [113]. GJs are also instrumental in conducting a hyperpolarizing, electrical wave between ECs that modulates the vascular tone [114,115]. The coupling of ECs with smooth muscle cells *via* myoendothelial junctions contributes to modulating the vessel diameter that is subject to changes in blood pressure, blood flow and shear stress [116–118]. Alterations in these parameters have furthermore been demonstrated to adjust Cx expression levels in ECs [119,120]. In capillaries, endothelial Cx channels have been associated with inflammatory responses. In lung alveolar blood vessels for instance, GJs mediate the bidirectional propagation of intercellular Ca^{2+} waves from capillaries to first order venules where the Ca^{2+} signal induces the expression of P-selectin, a cell-adhesion molecule that is important for leukocyte recruitment [121]. Similar results were reported for hamster cheek pouch capillaries [122] and capillaries of the renal cortex [120].

As for the CNS, there is no doubt about the presence of Cxs in the different vascular beds, but it is less well established which Cx subtypes are being expressed in the BBB. It is more or less accepted that brain microvascular endothelium expresses Cx37 and Cx40 [7,123–125] (Table 1). In addition, Cx43 was identified in freshly isolated capillary and microvascular ECs of bovine, rat, mouse and porcine brain [7,123,126–129]; however experiments performed on rat brain slices could not confirm such observations [30].

Several lines of evidence point to the possible contribution of endothelial Cxs in determining the permeability status of the BBB. Lum and colleagues indicate that Cx40 and Cx43 are associated with occludin and claudin-5 in porcine brain ECs, possibly *via* their association with ZO-1 [130]. Others have proposed a role of Cx channels in stabilizing brain endothelial junctions. Blocking Cx channels with 18β-glycyrrhetinic acid or oleamide, two non-specific Cx channel blockers, did not influence the expression or localization of TJ proteins, but did inhibit the barrier function of the TJs, based on measurements of TEER and paracellular flux of mannitol and inulin *in vitro* [7]. However, most knowledge on the role of Cxs in modulating the BBB comes from pathological models. In cerebral ischemia, a condition associated with disturbed BBB functionality, Cx43 immunolabelling was lost from isolated brain capillaries [131]. However, the authors indicate that glial endfeet are still present in their microvessel preparations, so care should be taken when ascribing the loss of Cx43 immunolabelling entirely to ECs. Another condition generally associated with a progressive dysfunction of the BBB is aging. Using ovariectomy of young animals as a menopause/aging model, it was shown that Cx43 redistributes in the BBB ECs without accompanying changes in ZO-1. Nevertheless, BBB permeability to Evans Blue was increased compared to non-ovariectomized controls [128]. These

Table 1
Cx expression in cells constituting the different CNS interfaces. For references and abbreviations: see text.

Barrier/interface	Cell type	Cx expression
Blood–brain interface	EC	Cx37 Cx40 Cx43
	Astrocyte	Cx26 Cx30 Cx43
	Pericyte	Cx37 Cx40 Cx43
Blood–retina interface	EC (iBRB)	Cx30.2 (murine orthologue of human Cx31.9) Cx37 Cx40 Cx43
	RPE (oBRB)	Cx40 Cx43 Cx46
	Müller cells	Cx43 Cx45
	Pericyte	Cx30.2 (murine orthologue of human Cx31.9) Cx37 Cx40 Cx43
BSCB	EC	Cx43
BCSFB	CPE	Cx23 Cx26 Cx30.3 Cx31.1 Cx36 Cx43 Cx50
	Tanycyte	Cx43
	Leptomeningeal cells	Cx26 Cx30 Cx43
Brain–CSF interface	Ependymal cells	Cx26 Cx30 Cx43 Cx45

data indicate that a loss in endothelial cell–cell communication due to altered hormonal levels, may underpin the barrier defects associated with ageing. CxHCs have been implicated in the inflammatory response of BBB cells. Exposure of immortalized rat brain RBE4 cells to conditioned medium harvested from lipopolysaccharide-activated microglia induces CxHC-mediated dye uptake in these ECs [108]. Treatment of immortalized mouse bEnd5 cells with peptidoglycan also activated Cx43 HCs. This led to the release of the extracellular messenger molecule ATP and resulted in increased expression of the toll-like receptor 2, potentiating the production of interleukin-6 [132]. Work from our hand brought up a novel role for CxHCs in the regulation of BBB function by the inflammatory stressor bradykinin. Here, CxHCs influenced BBB permeability *via* their involvement in the Ca^{2+} signaling machinery activated by bradykinin. The endothelial cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) is an important determinant of BBB function and an increase in endothelial $[Ca^{2+}]_i$ is invariably associated with a dysfunctional barrier (reviewed in [133]). CxHCs are Ca^{2+} permeable channels that open in response to a $[Ca^{2+}]_i$ increase, therefore contributing to Ca^{2+}-induced Ca^{2+} entry [134]. Additionally, open HCs mediate diffusive ATP release that subsequently engages in an autocrine signaling loop by activating purinergic receptors that activate phospholipase C (PLC) and promote the generation of inositol 1,4,5-trisphosphate (IP_3), triggering Ca^{2+} release from endoplasmic reticulum Ca^{2+} stores. $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{11}$ and $P2Y_{12}$ are present in BBB ECs [135–138]. Both CxHC-related mechanisms contribute to the oscillatory $[Ca^{2+}]_i$ changes induced by bradykinin and appear to be critical for Ca^{2+} oscillation-induced BBB dysfunction [129,134]. Notably, CxHC-supported endothelial Ca^{2+} oscillations were not associated with alterations in occludin and ZO-1 organization, suggesting that altered BBB function may be related to increased transcellular trafficking across BBB endothelial cells. The increase in $[Ca^{2+}]_i$ may be furthermore communicated to adjacent endothelial cells as intercellular Ca^{2+} waves [139], the propagation of which depends on GJs as well as CxHCs. GJs directly pass IP_3 to adjacent cells whereas CxHCs provide a paracrine ATP-dependent Ca^{2+} signaling route [140]. Our data indicate that the propagation of endothelial intercellular Ca^{2+} waves is associated with high amplitude $[Ca^{2+}]_i$ changes and a large permeability increase, which is more pronounced than the permeability increase associated with bradykinin-induced Ca^{2+} oscillations [139]. Taken together, these findings demonstrate that endothelial Ca^{2+} dynamics in concert with Cx channels influence BBB function, with CxHCs contributing to Ca^{2+} oscillations and CxHCs and GJs contributing to the spatial spread of $[Ca^{2+}]_i$ changes as intercellular Ca^{2+} waves [140].

The ECs of the inner retinal barrier express all vascular Cxs (Cx37, Cx40 and Cx43) as well as murine Cx30.2 that shows 84% similarity with human Cx31.9 [141–144] (Table 1). Recent evidence points toward the importance of Cxs in vascular network formation in the retina. RTEF-1 (related transcription enhancer factor-1) is a major player in the regulation of angiogenesis, the process during which ECs proliferate, migrate and form networks as primitive tubes mature into blood vessels. RTEF-1 was additionally identified as a prime regulator of Cx37, Cx40, and most importantly, Cx43 expression in retinal ECs. The knock-down of RTEF-1 and of Cx43 gave similar effect profiles characterized by acellular capillaries and disconnected vascular networks, indicating a role in EC aggregation rather than proliferation [90]. Although an increase in GJ-mediated intercellular communication was found to accompany the increase in Cx expression, it is unknown whether GJs are required for EC aggregation. Indeed, Cxs exert adhesive properties that are not related to their channel function [73–77]. Knock-out of Cx43 and Cx30.2 results in EC apoptosis and vascular lesions featured as acellular capillaries and pericyte loss in the retinal tissue [142,145]. Exposure of cultured retinal ECs to high glucose (30 mM) or injection of streptozotocin, mimicking diabetic retinopathy respectively *in vitro* and *in vivo*, has similar effects. Apoptosis does not simply result from a loss of Cx43, but from a defective intercellular communication since Cx channel block equally gives rise to EC death [144]. Different hypotheses exist to explain the link between loss of cell–cell communication and apoptosis. These include the impeded exchange of survival or rescue signals as well as a compromised spatial clearing of toxic molecules [146]. Interestingly, high glucose also reduced Cx43 levels in the endothelial mitochondria [147]. Cx43 has been recently revealed in the inner mitochondrial membrane of various cell types, but its role remains mostly speculative. The downregulation of mitochondrial Cx43 in retinal ECs is accompanied by a fragmentation of the mitochondrial network and release of the apoptotic mediator Cytochrome C [147]. In cells not experiencing cell death, the reduction in Cx43 expression is accompanied by a downregulation of ZO-1 and occludin, and hence, compromised TJs. The exogenous expression of Cx43 in these conditions protects the cells from high glucose-induced changes in occludin and ZO-1 and prevents an increase in monolayer permeability [141]. Conversely, the loss of retinal ECs observed with ischemia/reperfusion is ascribed to an upregulation of Cx43 [148]. It is hypothesized that Cx43HCs open as a result of deficient supply of oxygen and glucose, contributing to EC death. Vessel rupture, dye leakage, astrogliosis and retinal ganglion cell loss accompanied the EC loss. Similar

observations were also made in ischemic, excised optic nerves [149]. Partial optic nerve transection, as a model for traumatic injury, had no effect on Cx43 expression in the microvessel, but unfortunately, Cx channel function was not investigated in the ECs [150].

Finally, in a rodent model for traumatic spinal cord injury, a significant elevation of Cx43 expression was observed in the walls of small blood vessels, as early as 6 h after injury. This upregulation was accompanied by vascular leakage of fluorescently-labelled albumin and accumulation of blood–borne neutrophils. Preventing Cx43 upregulation using antisense oligodeoxynucleotides, overcame the vascular leak and neutrophil recruitment to the vessel wall [151].

3.2. Cxs in the endothelial cell partners of the blood–brain interface

As described in chapter 1, the BBB is surrounded by barrier-supportive astrocytes and pericytes that have an indisputable, be it direct or trophic, influence on the barrier forming endothelium. Astrocytes and pericytes have been far better characterized in terms of Cx expression and Cx channel function as compared to the ECs.

Astrocytes form extensive networks through which they dilute glutamate and potassium ions taken up from the extracellular space during neuronal action potential firing and synaptic activity. In this way they prevent a build-up of potassium and glutamate, protecting neurons from excitotoxic death. Through the release of gliotransmitters they additionally modulate synaptic transmission while at the same time, astrocytes physically and functionally connect neuronal synapses with the cerebral vasculature, helping to adjust blood flow to metabolic demand. In all of these functions, glial GJs and CxHCs, mostly made up of Cx30 and Cx43 (Table 1), have been shown to fulfil important tasks [110]. In the retina, Cx43 and Cx45 expressed in macroglial Müller cells seem to support comparable functions [152–154]. Despite the abundance of Cxs in astrocytes and their well-known role in neuronal functioning, little is known on how glial, perivascular Cxs would contribute to the endothelial barriers of the CNS. Glial endfeet enriched in Cx30 and Cx43 appear around BBB capillaries 15–20 days after birth, i.e. when the BBB is functional [155]. Under normal circumstances, $Cx30^{-/-}$ $Cx43^{fl/fl}$/hGFAP-Cre dKO mice that have global Cx30 knock-out combined with a glial-specific deletion of Cx43, also display normal TJ morphology between ECs, and the BBB is functionally intact. However, when vascular pressure is increased, BBB permeability is largely elevated compared to wild type mice [155]. Thus, it is suggested that glial endfeet Cxs strengthen the barrier and preserve barrier function in case of stress.

Pericytes are vascular mural cells that share the basement membrane with capillary ECs [156,157]. They are multifunctional cells endowed with the regulation of endothelial TJ/AJ development (see above), vascular stability and architecture, extracellular matrix secretion, vessel diameter and they exhibit phagocytic properties [158]. In areas where the basement membrane is absent, pericytes physically contact the EC monolayer *via* so called peg-and-socket junctions (PSJs); reciprocal extrusions from one cell that invaginate the other cell, leaving an intercellular gap of ~20 nm [159,160]. In comparison, the intercellular cleft at the level of GJ plaques amounts only 3.5 nm [161]; therefore, direct cytoplasmic communication at the site of PSJs is inconceivable. Nevertheless, PSJs have been suggested to contain Cx channels [158,159], which are most likely CxHCs that enable paracrine signaling at this site. The primary building block of the pericytic CxHCs is Cx43, but Cx37, Cx40 and Cx30.2 have also been identified [142,162] (Table 1). Functionally, PSJs have been implicated in electric field coupling [159] and paracrine growth factor signaling [157]. Outside PSJs, pericytes may form GJs with ECs since the differentiation of mesenchymal cells into pericytes during embryonic development is initiated by direct junctional contact with ECs that leads to TGFβ signaling [163]. Pericytes also respond to vasoconstrictors by an increase in $[Ca^{2+}]_i$ *via* GJs. This Ca^{2+} signal is further propagated to smooth muscle cells that surround higher-order arterioles and venules [164].

3.3. Cxs at the epithelial BCSFB and oBRB

Compared to the recently reviving interest in Cxs in endothelial cerebral barriers, hardly anything is known on the role of Cxs in the cells that constitute the blood–CSF interface.

In choroid plexus preparations consisting primarily of CPE (90% purity), microarray analysis identified genes coding for Cx23, Cx30.3, Cx31.1, Cx36 and Cx43 (Table 1). Note however, that although ependymal cells constitute only a minority in this preparation, it cannot be excluded that the detection of Cxs in CPE by qPCR is biased by the ependymal cell fraction. The expression of these genes varied from the embryonic phase up to adulthood, with Cx43 being the most relevant in adult tissue [165]. Non-punctate expression of Cx50 as well as small and rare Cx26 *punctae* were additionally identified in the CPE [125,166]. It has been suggested that Cx43 in the CPE contributes to the autoimmune response that forms the basis of myelin degradation in multiple sclerosis. Upon induction of experimental autoimmune encephalomyelitis (EAE, a mouse model of multiple sclerosis), the CPE undergoes numerous morphological and functional changes, including the reorganization of claudins [115]. In addition, EAE potentiates the expression of Cx43 in CPE and increases the thickness of GJ plaques in atomic force microscopy [167]. It is however unknown how this relates to the progression of the disease.

Tanycytes have received a substantial interest related to their Cx expression and function. Recent evidence suggests that tanycytes, similar to glial cells, signal among each other *via* changes in $[Ca^{2+}]_i$ rather than through changes in membrane potential. The cells respond to several extracellular messengers, including ATP, histamine and acetylcholine, by exhibiting a rapid and robust $[Ca^{2+}]_i$ increase that can propagate to neighboring cells with a velocity similar to the propagation of intercellular Ca^{2+} waves in other cell types. Tanycytes may also release ATP that functions as a paracrine messenger for the propagation of Ca^{2+} waves. Thus, it is plausible that tanycytes function, like astrocytes, as a network coordinated by GJs and paracrine purinergic signaling [168–170]. Cx43 has been identified in tanycytes (Table 1), but there is no consensus yet in which of the tanycyte subclasses it resides. Whereas some find expression of Cx43 only in $alpha_1$ tanycytes [61], others identify Cx43 in $beta_1$ cells [171]. Consistent with older data [172], these $beta_1$ cells effectively transfer micro-injected Lucifer yellow to neighboring cells *via* GJs. They also take up the small MW dye ethidium bromide when CxHC opening is stimulated by omission of extracellular divalent ions in the bathing solution. Generally taken, tanycytes are thus equipped with functional GJs and CxHCs. Cx43HCs were proposed to play a role in the glucosensing capacity of the $beta_1$ tanycytes. Glucose is taken up by tanycytes through glucose transporters and is converted to ATP through glycolysis. ATP is then released *via* Cx43HCs and acts in an autocrine manner to stimulate an increase in $[Ca^{2+}]_i$ [171], similar to observations made in BBB ECs [129]. The increase in $[Ca^{2+}]_i$ may further potentiate CxHC opening so that they can contribute to glucose uptake. Whether or not GJ channels are involved in the propagation of glucose or Ca^{2+} waves to neighboring tanycytes remains to be determined. ATP release by tanycytes may modulate neuronal activity in hypothalamic areas associated with feeding behavior. On the other hand, it was also demonstrated that blood glucose levels do not differ between wild type and heterozygous $Cx43^{+/-}$ mice [145], suggesting that Cx43 downregulation may not

interfere with the overall glucosensing hypothalamic function. However, residual expression in the heterozygous Cx43$^{+/-}$ mice model may be sufficient to support hypothalamic function, precluding strong conclusions to be made.

Finally, GJs are abundant in developing and adult meninges [173–176] with adult meningeal fibroblasts displaying intense immunoreactivity for Cx26, Cx30 and Cx43 [166,174,177,178] (Table 1). Cx26 has additionally been identified in leptomeningeal cells [135,174], i.e., cells derived from the pial and arachnoid membrane with fibroblast-like appearance. Meningeal projections reach the lateral ventricles and the roof of the third ventricle and are continuous with the stroma of the choroid plexus that is separated from the CPE by a basal lamina [166]. Rat brain tissue sections reveal abundant Cx26 *punctae* in these meningeal projections and in the choroid plexus stroma [166]. Cx26 and Cx30 may form heterotypic channels and in Cx30 knock-out mice, where Cx30 is deleted from the leptomeninges, Cx26 was lost as well [136]. Leptomeningeal Cx43 expression was unaltered in Cx30 knock-out mice, but has been shown to disappear upon trypanosome infection. Cx43 was deleted only from those cells that were infiltrated with the parasites [117], but unfortunately, the functional implications of this loss in Cx43 expression remain unknown.

RPE cells of the oBRB, that prevent choroidal blood from accessing the neural retina (rods and cones), have been shown to contain mRNA of Cx26, Cx32, Cx36, Cx43, Cx45 and Cx46, but of these, only Cx43 and Cx46 appear at protein level [179]. An independent study also identified Cx40 in RPE cells [180] (Table 1). GJs in the RPE cell layer have been shown to be important for the secretion of the aqueous humor that fills the anterior and posterior chamber of the eye [180]. GJs are also implied in the spread of CytC-induced apoptosis in the RPE [181] and in the production of the pro-angiogenic factor VEGF (vascular endothelial growth factor) [182]. Finally, ATP release from the RPE and the subsequent spread of intercellular Ca^{2+} waves between RPEs is strictly dependent on GJs and HCs composed of Cx43 and are indispensable for the development of the neural retina (i.e., rods and cones) [183]. With respect to a Cx contribution to the barrier function of the RPE, fairly little is known. Cx43 knock-out in RPE has been associated with leakiness of the BRB towards HRP [180]; yet, TJs remained intact in this study suggesting other pathways like transcytosis. Further work will thus be necessary to explain the contribution of Cxs in the RPE barrier.

3.4. Cxs in the ependymal brain/CSF interface

Ependymal cells are situated between the brain and CSF but are not barrier forming cells, allowing direct contact between the CSF and the brain interstitial compartment. Ependymal cells are well-coupled with one another and with underlying glial cells; GJs can be made up of Cx26, Cx30, Cx43 or Cx45, all of which have been identified in ependymal cells [53,138,156,184,185] (Table 1). Ependymal cells secrete a multitude of growth factors via which they have been suggested to play a part in the organization of the subventricular zone [53] and GJs may act to synchronize ependymal cells in this process. Additionally, GJs assist in synchronizing the beating of cilia which is important for bulk CSF flow [156]. Aquaporin-4 deletion in ependymal cells has been shown to result in the disappearance of Cx43 and in reduced CSF production, diminished ventricle volume and increased brain water content [186]; however, whether this is directly related to aquaporin-4 knock-down or Cx43 deficiency is uncertain. Importantly, a subset of ependymal cells located in the central canal of the spinal cord have been ascribed stem cell properties. These cells are activated upon spinal cord injury and proliferate towards the lesion site where they differentiate into new neurons and oligodendrocytes, or into new astrocytes, forming a glial scar [187,188]. GJs may contribute to their transformation since they stimulate stem cell proliferation [189]. Recent investigations of these specialized ependymal cells indicate that they express Cxs, including Cx45, and that they are well-coupled [53,158,187,190].

4. Pannexin channels

Pannexins (Panxs) constitute a second class of vertebrate, HC forming proteins [191,192]. In the mouse and human genomes, 3 Panx genes, *PANX1*, *PANX2* and *PANX3* were described [170]. Unlike Cxs, Panxs are highly glycosylated on the extracellular domains, which negatively affects docking and GJ channel formation [159–161]; Panxs are therefore suggested to only form HCs. Indeed, today, ample evidence continues to support a role for PanxHCs in a variety of cells and tissues [57].

Panx1 is ubiquitously expressed whereas Panx2 is confined to the CNS. The expression of these Panxs in the cerebral vasculature was only revealed recently [193]; Panx1 and Panx2 are expressed in the rat middle cerebral artery where Panx1 was identified in smooth muscle cells only, and Panx2 in ECs and smooth muscle cells. Panx1 was additionally identified in bEnd5 brain endothelial cells [132]. Using microarray analysis, *PANX1* was also identified in CPE [165]. Maslieieva and Thompson have recently revealed that ATP release through Panx1 channels in the CPE recruits epiplexus cells that are part of the central innate immune system [193]. In tanycytes, where CxHCs are involved in the intracellular Ca^{2+} response to increased levels of extracellular glucose (see above), Panx1 channel blockers had no effect [171]. These data at least indicate that Panx1 is not involved in the process; however, it does not rule out a possible expression of Panx1 in tanycytes. Unfortunately, up to date, we have no knowledge of any other reports that pinpoint Panx expression and function in the barrier-forming cells of the CNS, including BRB and BBB ECs, CPE and tanycytes.

5. Conclusions

Ion and metabolite homeostasis in the nervous tissue is strictly controlled by a series of barriers interposed between the blood, CSF and interstitial fluid. The BBB, iBRB and BSCB are endothelial barriers that prevent the direct contact between the circulation and the nervous tissue at the level of the brain, retina and spinal cord, whereas an epithelial BCSFB wraps around the fenestrated, leaky vessels in ventricles, meninges and secretory organs to shield-off the blood from the CSF. TJs and AJs, that come together in a junctional complex physically linking endothelial and epithelial cells, are considered the main structural components of the barriers. However, a third junctional partner, the Cx channels, are largely overlooked. These proteins may not form true diffusion barriers like TJs and AJs, but by forming CxHCs and GJs they may have important regulatory functions. CxHCs allow contact between the cytosol and the extracellular space whereas GJs, connective channels formed by two docked CxHCs, allow direct contact between the cytoplasms of adjacent cells. These channels thus allow for autocrine and paracrine signaling as well as the direct transfer of nutrients and metabolites, second messengers, ions and signaling molecules. Recent evidence, mostly coming from the endothelial barriers, indicates that Cx channels are involved in EC aggregation, adhesion and apoptosis and that they may regulate barrier permeability in inflammatory conditions. The role of Cx channels in the BCSFB remains however largely unexplored. In addition, there is a large gap in our knowledge regarding the potential role of Cx channels in signaling between the barrier forming cells and the cells that surround them, i.e. astrocytes and pericytes, that express high levels of Cxs and are known to exert strong influence on

barrier tightness. We conclude that Cxs are an unexplored and interesting target involved in barrier function as well as dysfunction.

Acknowledgements

This work was supported by the Fund for Scientific Research Flanders (FWO – Grant numbers G.0354.07, G.0140.08 and 3G.0134.09 to L. Leybaert; 3G035113N, 31517710N and 31500313N to R.E. Vandenbroucke) and the Interuniversity Attraction Poles Program (Belgian Science Policy Projects P6/31 and P7/10 to L. Leybaert). Figures have been produced using Servier Medical Art.

References

[1] Abbott, N.J., Patabendige, A.A., Dolman, D.E., Yusof, S.R. and Begley, D.J. (2010) Structure and function of the blood–brain barrier. Neurobiol. Dis. 37, 13–25.
[2] Bechmann, I., Galea, I. and Perry, V.H. (2007) What is the blood–brain barrier (not)? Trends Immunol. 28 (1), 5–11.
[3] Ge, S., Song, L. and Pachter, J.S. (2005) Where is the blood–brain barrier ... really? J. Neurosci. Res. 79, 421–427.
[4] Wolburg, H. and Lippoldt, A. (2002) Tight junctions of the blood–brain barrier: development, composition and regulation. Vascul. Pharmacol. 38, 323–337.
[5] Bradbury, M.W. (1993) The blood–brain barrier. Exp. Physiol. 78, 453–472.
[6] Dejana, E. (2004) Endothelial cell–cell junctions: happy together. Nat. Rev. Mol. Cell Biol. 5, 261–270.
[7] Nagasawa, K., Chiba, H., Fujita, H., Kojima, T., Saito, T., Endo, T. and Sawada, N. (2006) Possible involvement of gap junctions in the barrier function of tight junctions of brain and lung endothelial cells. J. Cell. Physiol. 208, 123–132.
[8] Ek, C.J., Dziegielewska, K.M., Stolp, H. and Saunders, N.R. (2006) Functional effectiveness of the blood–brain barrier to small water-soluble molecules in developing and adult opossum (Monodelphis domestica). J. Comp. Neurol. 496, 13–26.
[9] Ribatti, D., Nico, B., Crivellato, E. and Artico, M. (2006) Development of the blood–brain barrier: a historical point of view. Anat. Rec. B New Anat. 289, 3–8.
[10] Furuse, M., Sasaki, H. and Tsukita, S. (1999) Manner of interaction of heterogeneous claudin species within and between tight junction strands. J. Cell Biol. 147, 891–903.
[11] Nitta, T., Hata, M., Gotoh, S., Seo, Y., Sasaki, H., Hashimoto, N., Furuse, M. and Tsukita, S. (2003) Size-selective loosening of the blood–brain barrier in claudin-5-deficient mice. J. Cell Biol. 161, 653–660.
[12] Lippoldt, A., Liebner, S., Andbjer, B., Kalbacher, H., Wolburg, H., Haller, H. and Fuxe, K. (2000) Organization of choroid plexus epithelial and endothelial cell tight junctions and regulation of claudin-1, -2 and -5 expression by protein kinase C. NeuroReport 11, 1427–1431.
[13] Cardoso, F.L., Brites, D. and Brito, M.A. (2010) Looking at the blood–brain barrier: molecular anatomy and possible investigation approaches. Brain Res. Rev. 64, 328–363.
[14] Furuse, M., Hirase, T., Itoh, M., Nagafuchi, A., Yonemura, S. and Tsukita, S. (1993) Occludin: a novel integral membrane protein localizing at tight junctions. J. Cell Biol. 123, 1777–1788.
[15] Saitou, M., Furuse, M., Sasaki, H., Schulzke, J.D., Fromm, M., Takano, H., Noda, T. and Tsukita, S. (2000) Complex phenotype of mice lacking occludin, a component of tight junction strands. Mol. Biol. Cell 11, 4131–4142.
[16] Furuse, M., Sasaki, H., Fujimoto, K. and Tsukita, S. (1998) A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. J. Cell Biol. 143, 391–401.
[17] McCarthy, K.M., Skare, I.B., Stankewich, M.C., Furuse, M., Tsukita, S., Rogers, R.A., Lynch, R.D. and Schneeberger, E.E. (1996) Occludin is a functional component of the tight junction. J. Cell Sci. 109 (Pt 9), 2287–2298.
[18] Davies, D.C. (2002) Blood–brain barrier breakdown in septic encephalopathy and brain tumours. J. Anat. 200, 639–646.
[19] Kratzer, I., Vasiljevic, A., Rey, C., Fevre-Montange, M., Saunders, N., Strazielle, N. and Ghersi-Egea, J.F. (2012) Complexity and developmental changes in the expression pattern of claudins at the blood–CSF barrier. Histochem. Cell Biol. 138, 861–879.
[20] Hirase, T. et al. (1997) Occludin as a possible determinant of tight junction permeability in endothelial cells. J. Cell Sci. 110 (Pt 14), 1603–1613.
[21] Martin-Padura, I. et al. (1998) Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. J. Cell Biol. 142, 117–127.
[22] Ebnet, K., Suzuki, A., Ohno, S. and Vestweber, D. (2004) Junctional adhesion molecules (JAMs): more molecules with dual functions? J. Cell Sci. 117, 19–29.
[23] Bednarczyk, J. and Lukasiuk, K. (2011) Tight junctions in neurological diseases. Acta Neurobiol. Exp. (Wars) 71, 393–408.
[24] Liu, W.Y., Wang, Z.B., Zhang, L.C., Wei, X. and Li, L. (2012) Tight junction in blood–brain barrier: an overview of structure, regulation, and regulator substances. CNS Neurosci. Ther. 18, 609–615.
[25] Saunders, N.R., Ek, C.J., Habgood, M.D. and Dziegielewska, K.M. (2008) Barriers in the brain: a renaissance? Trends Neurosci. 31, 279–286.
[26] Butt, A.M., Jones, H.C. and Abbott, N.J. (1990) Electrical resistance across the blood–brain barrier in anaesthetized rats: a developmental study. J. Physiol. 429, 47–62.
[27] Saunders, N.R., Habgood, M.D. and Dziegielewska, K.M. (1999) Barrier mechanisms in the brain, II. Immature brain. Clin. Exp. Pharmacol. Physiol. 26, 85–91.
[28] Engelhardt, B. (2003) Development of the blood–brain barrier. Cell Tissue Res. 314, 119–129.
[29] Lok, J., Gupta, P., Guo, S., Kim, W.J., Whalen, M.J., van Leyen, K. and Lo, E.H. (2007) Cell–cell signaling in the neurovascular unit. Neurochem. Res. 32, 2032–2045.
[30] Simard, M., Arcuino, G., Takano, T., Liu, Q.S. and Nedergaard, M. (2003) Signaling at the gliovascular interface. J. Neurosci. 23, 9254–9262.
[31] Bauer, H.C. and Bauer, H. (2000) Neural induction of the blood–brain barrier: still an enigma. Cell. Mol. Neurobiol. 20, 13–28.
[32] Kniesel, U., Risau, W. and Wolburg, H. (1996) Development of blood–brain barrier tight junctions in the rat cortex. Brain Res. Dev. Brain Res. 96, 229–240.
[33] Hirase, H., Creso, J., Singleton, M., Bartho, P. and Buzsaki, G. (2004) Two-photon imaging of brain pericytes *in vivo* using dextran-conjugated dyes. Glia 46, 95–100.
[34] Shepro, D. and Morel, N.M. (1993) Pericyte physiology. FASEB J. 7, 1031–1038.
[35] Berezowski, V., Landry, C., Dehouck, M.P., Cecchelli, R. and Fenart, L. (2004) 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.
[36] Lai, C.H. and Kuo, K.H. (2005) The critical component to establish *in vitro* BBB model: Pericyte. Brain Res. Brain Res. Rev. 50, 258–265.
[37] Ramsauer, M., Krause, D. and Dermietzel, R. (2002) Angiogenesis of the blood–brain barrier *in vitro* and the function of cerebral pericytes. FASEB J. 16, 1274–1276.
[38] Hellstrom, M., Gerhardt, H., Kalen, M., Li, X., Eriksson, U., Wolburg, H. and Betsholtz, C. (2001) Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. J. Cell Biol. 153, 543–553.
[39] Nakagawa, S., Deli, M.A., Nakao, S., Honda, M., Hayashi, K., Nakaoke, R., Kataoka, Y. and Niwa, M. (2007) Pericytes from brain microvessels strengthen the barrier integrity in primary cultures of rat brain endothelial cells. Cell. Mol. Neurobiol. 27, 687–694.
[40] Dohgu, S. et al. (2005) Brain pericytes contribute to the induction and up-regulation of blood–brain barrier functions through transforming growth factor-beta production. Brain Res. 1038, 208–215.
[41] Hori, S., Ohtsuki, S., Hosoya, K., Nakashima, E. and Terasaki, T. (2004) 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.
[42] Tong, X.K. and Hamel, E. (2007) Transforming growth factor-beta1 impairs endothelin-1-mediated contraction of brain vessels by inducing mitogen-activated protein (MAP) kinase phosphatase-1 and inhibiting p38 MAP kinase. Mol. Pharmacol. 72, 1476–1483.
[43] Zhou, M. et al. (1998) Fibroblast growth factor 2 control of vascular tone. Nat. Med. 4, 201–207.
[44] Fischer, M. et al. (2011) Angiopoietin-1 is associated with cerebral vasospasm and delayed cerebral ischemia in subarachnoid hemorrhage. BMC Neurol. 11, 59.
[45] Johanson, C.E. (2008) Choroid Plexus-Cerebrospinal Fluid Circulatory Dynamics: Impact on Brain Growth, Metabolism, and Repair in: (Conn, P.M., Ed.), pp. 173–200, Humana Press, Portland, Oregon.
[46] Redzic, Z. (2011) Molecular biology of the blood–brain and the blood–cerebrospinal fluid barriers: similarities and differences. Fluids Barriers CNS 8, 3.
[47] Saito, Y. and Wright, E.M. (1984) Regulation of bicarbonate transport across the brush border membrane of the bull-frog choroid plexus. J. Physiol. 350, 327–342.
[48] Shechter, R., London, A. and Schwartz, M. (2013) Orchestrated leukocyte recruitment to immune-privileged sites: absolute barriers versus educational gates. Nat. Rev. Immunol. 13, 206–218.
[49] Strazielle, N. and Ghersi-Egea, J.F. (2013) Physiology of blood–brain interfaces in relation to brain disposition of small compounds and macromolecules. Mol. Pharm. 10, 1473–1491.
[50] Vandenbroucke, R.E. et al. (2012) Matrix metalloprotease 8-dependent extracellular matrix cleavage at the blood–CSF barrier contributes to lethality during systemic inflammatory diseases. J. Neurosci. 32, 9805–9816.
[51] Marques, F. et al. (2009) Kinetic profile of the transcriptome changes induced in the choroid plexus by peripheral inflammation. J. Cereb. Blood Flow Metab. 29, 921–932.
[52] Marques, F. et al. (2011) Transcriptome signature of the adult mouse choroid plexus. Fluids Barriers CNS 8, 10.
[53] Del Bigio, M.R. (2010) Ependymal cells: biology and pathology. Acta Neuropathol. 119, 55–73.

[54] Dermietzel, R. (1975) Junctions in the central nervous system of the cat. IV. Interendothelial junctions of cerebral blood vessels from selected areas of the brain. Cell Tissue Res. 164, 45–62.
[55] Somjen, G.G. (2004) The Regulation of Ions in the BrainIons in the Brain. Normal Function, Seizures, and Stroke, pp. 13–62, Oxford University Press.
[56] Leybaert, L. (2005) Neurobarrier coupling in the brain: a partner of neurovascular and neurometabolic coupling? J. Cereb. Blood Flow Metab. 25, 2–16.
[57] Leen, W.G., Willemsen, M.A., Wevers, R.A. and Verbeek, M.M. (2012) Cerebrospinal fluid glucose and lactate: age-specific reference values and implications for clinical practice. PLoS ONE 7, e42745.
[58] Peruzzo, B., Pastor, F.E., Blazquez, J.L., Schobitz, K., Pelaez, B., Amat, P. and Rodriguez, E.M. (2000) A second look at the barriers of the medial basal hypothalamus. Exp. Brain Res. 132, 10–26.
[59] Mullier, A., Bouret, S.G., Prevot, V. and Dehouck, B. (2010) Differential distribution of tight junction proteins suggests a role for tanycytes in blood–hypothalamus barrier regulation in the adult mouse brain. J. Comp. Neurol. 518, 943–962.
[60] Langlet, F., Mullier, A., Bouret, S.G., Prevot, V. and Dehouck, B. (2013) Tanycyte-like cells form a blood–cerebrospinal fluid barrier in the circumventricular organs of the mouse brain. J. Comp. Neurol. 521, 3389–3405.
[61] Rodriguez, E.M., Blazquez, J.L. and Guerra, M. (2010) The design of barriers in the hypothalamus allows the median eminence and the arcuate nucleus to enjoy private milieus: the former opens to the portal blood and the latter to the cerebrospinal fluid. Peptides 31, 757–776.
[62] Engelhardt, B. and Sorokin, L. (2009) The blood–brain and the blood–cerebrospinal fluid barriers: function and dysfunction. Semin. Immunopathol. 31, 497–511.
[63] Segal, M.B. (2000) The choroid plexuses and the barriers between the blood and the cerebrospinal fluid. Cell. Mol. Neurobiol. 20, 183–196.
[64] Cipolla, M.J. (2009) The Cerebral Circulation, Morgan & Claypool Life Sciences, San Rafael (CA).
[65] Saunders, N.R., Daneman, R., Dziegielewska, K.M. and Liddelow, S.A. (2013) Transporters of the blood–brain and blood–CSF interfaces in development and in the adult. Mol. Aspects Med. 34, 742–752.
[66] Cunha-Vaz, J., Bernardes, R. and Lobo, C. (2011) Blood–retinal barrier. Eur. J. Ophthalmol. 21 (Suppl. 6), S3–S9.
[67] Rizzolo, L.J., Peng, S., Luo, Y. and Xiao, W. (2011) Integration of tight junctions and claudins with the barrier functions of the retinal pigment epithelium. Prog. Retin. Eye Res. 30, 296–323.
[68] Bartanusz, V., Jezova, D., Alajajian, B. and Digicaylioglu, M. (2011) The blood–spinal cord barrier: morphology and clinical implications. Ann. Neurol. 70, 194–206.
[69] Derangeon, M., Spray, D.C., Bourmeyster, N., Sarrouilhe, D. and Herve, J.C. (2009) Reciprocal influence of connexins and apical junction proteins on their expressions and functions. Biochim. Biophys. Acta 1788, 768–778.
[70] Meyer, R.A., Laird, D.W., Revel, J.P. and Johnson, R.G. (1992) Inhibition of gap junction and adherens junction assembly by connexin and A-CAM antibodies. J. Cell Biol. 119, 179–189.
[71] Beyer, E.C., Paul, D.L. and Goodenough, D.A. (1987) Connexin43: a protein from rat heart homologous to a gap junction protein from liver. J. Cell Biol. 105, 2621–2629.
[72] Laird, D.W. (2006) Life cycle of connexins in health and disease. Biochem. J. 394, 527–543.
[73] Elias, L.A., Wang, D.D. and Kriegstein, A.R. (2007) Gap junction adhesion is necessary for radial migration in the neocortex. Nature 448, 901–907.
[74] Cina, C., Maass, K., Theis, M., Willecke, K., Bechberger, J.F. and Naus, C.C. (2009) Involvement of the cytoplasmic C-terminal domain of connexin43 in neuronal migration. J. Neurosci. 29, 2009–2021.
[75] Prochnow, N. and Dermietzel, R. (2008) Connexons and cell adhesion: a romantic phase. Histochem. Cell Biol. 130, 71–77.
[76] Bao, B., Jiang, J., Yanase, T., Nishi, Y. and Morgan, J.R. (2011) Connexon-mediated cell adhesion drives microtissue self-assembly. FASEB J. 25, 255–264.
[77] Cotrina, M.L., Lin, J.H. and Nedergaard, M. (2008) Adhesive properties of connexin hemichannels. Glia 56, 1791–1798.
[78] Bruzzone, R., White, T.W. and Paul, D.L. (1996) Connections with connexins: the molecular basis of direct intercellular signaling. Eur. J. Biochem. 238, 1–27.
[79] Falk, M.M. (2000) Connexin-specific distribution within gap junctions revealed in living cells. J. Cell Sci. 113 (Pt 22), 4109–4120.
[80] Gaietta, G. et al. (2002) Multicolor and electron microscopic imaging of connexin trafficking. Science 296, 503–507.
[81] Stout, C.E., Costantin, J.L., Naus, C.C. and Charles, A.C. (2002) Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels. J. Biol. Chem. 277, 10482–10488.
[82] Retamal, M.A., Cortes, C.J., Reuss, L., Bennett, M.V. and Saez, J.C. (2006) S-nitrosylation and permeation through connexin43 hemichannels in astrocytes: induction by oxidant stress and reversal by reducing agents. Proc. Natl. Acad. Sci. USA 103, 4475–4480.
[83] Retamal, M.A., Schalper, K.A., Shoji, K.F., Bennett, M.V. and Saez, J.C. (2007) Opening of connexin43 hemichannels is increased by lowering intracellular redox potential. Proc. Natl. Acad. Sci. USA 104, 8322–8327.
[84] Retamal, M.A., Froger, N., Palacios-Prado, N., Ezan, P., Saez, P.J., Saez, J.C. and Giaume, C. (2007) Cx43 hemichannels and gap junction channels in astrocytes are regulated oppositely by proinflammatory cytokines released from activated microglia. J. Neurosci. 27, 13781–13792.
[85] Ramachandran, S., Xie, L.H., John, S.A., Subramaniam, S. and Lal, R. (2007) A novel role for connexin hemichannel in oxidative stress and smoking-induced cell injury. PLoS ONE 2, e712.
[86] Contreras, J.E. et al. (2002) Metabolic inhibition induces opening of unapposed connexin43 gap junction hemichannels and reduces gap junctional communication in cortical astrocytes in culture. Proc. Natl. Acad. Sci. USA 99, 495–500.
[87] Bargiotas, P., Monyer, H. and Schwaninger, M. (2009) Hemichannels in cerebral ischemia. Curr. Mol. Med. 9, 186–194.
[88] Siller-Jackson, A.J., Burra, S., Gu, S., Xia, X., Bonewald, L.F., Sprague, E. and Jiang, J.X. (2008) Adaptation of connexin43-hemichannel prostaglandin release to mechanical loading. J. Biol. Chem. 283, 26374–26382.
[89] Stehberg, J. et al. (2012) Release of gliotransmitters through astroglial connexin43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. FASEB J. 26, 3649–3657.
[90] Wang, N. et al. (2013) Paracrine signaling through plasma membrane hemichannels. Biochim. Biophys. Acta 1828, 35–50.
[91] Chandrasekhar, A. and Bera, A.K. (2012) Hemichannels: permeants and their effect on development, physiology and death. Cell Biochem. Funct. 30, 89–100.
[92] Eugenin, E.A., Basilio, D., Saez, J.C., Orellana, J.A., Raine, C.S., Bukauskas, F., Bennett, M.V. and Berman, J.W. (2012) The role of gap junction channels during physiologic and pathologic conditions of the human central nervous system. J. Neuroimmune Pharmacol. 7, 499–518.
[93] Bennett, M.V., Garre, J.M., Orellana, J.A., Bukauskas, F.F., Nedergaard, M. and Saez, J.C. (2012) Connexin and pannexin hemichannels in inflammatory responses of glia and neurons. Brain Res. 1487, 3–15.
[94] Li, M.W., Mruk, D.D. and Cheng, C.Y. (2012) Gap junctions and blood–tissue barriers. Adv. Exp. Med. Biol. 763, 260–280.
[95] Kojima, T., Murata, M., Go, M., Spray, D.C. and Sawada, N. (2007) Connexins induce and maintain tight junctions in epithelial cells. J. Membr. Biol. 217, 13–19.
[96] E.E. Schneeberger, L.R. Martinez, Ultrastructure and immunolabeling of the tight junction, in: J.M. Anderson, M. Cereijido, (Eds.), Tight junctions, CRC Press, 2001, pp. 19–37. http://dx.doi.org/10.1201/9781420038538.ch2.
[97] Nusrat, A., Chen, J.A., Foley, C.S., Liang, T.W., Tom, J., Cromwell, M., Quan, C. and Mrsny, R.J. (2000) The coiled-coil domain of occludin can act to organize structural and functional elements of the epithelial tight junction. J. Biol. Chem. 275, 29816–29822.
[98] Wei, C.J., Francis, R., Xu, X. and Lo, C.W. (2005) Connexin43 associated with an N-cadherin-containing multiprotein complex is required for gap junction formation in NIH3T3 cells. J. Biol. Chem. 280, 19925–19936.
[99] Wu, J.C., Tsai, R.Y. and Chung, T.H. (2003) Role of catenins in the development of gap junctions in rat cardiomyocytes. J. Cell. Biochem. 88, 823–835.
[100] Gourdie, R.G., Ghatnekar, G.S., O'Quinn, M., Rhett, M.J., Barker, R.J., Zhu, C., Jourdan, J. and Hunter, A.W. (2006) The unstoppable connexin43 carboxyl-terminus: new roles in gap junction organization and wound healing. Ann. N. Y. Acad. Sci. 1080, 49–62.
[101] Duffy, H.S., Delmar, M. and Spray, D.C. (2002) Formation of the gap junction nexus: binding partners for connexins. J. Physiol. Paris 96, 243–249.
[102] Morita, H., Katsuno, T., Hoshimoto, A., Hirano, N., Saito, Y. and Suzuki, Y. (2004) Connexin26-mediated gap junctional intercellular communication suppresses paracellular permeability of human intestinal epithelial cell monolayers. Exp. Cell Res. 298, 1–8.
[103] Wan, H.T., Mruk, D.D., Wong, C.K. and Cheng, C.Y. (2014) Perfluorooctanesulfonate (PFOS) perturbs male rat Sertoli cell blood–testis barrier function by affecting F-actin organization via p-FAK-Tyr407 – an *in vitro* study. Endocrinology 155 (1), 249–262.
[104] Lohmann, C., Huwel, S. and Galla, H.J. (2002) Predicting blood–brain barrier permeability of drugs: evaluation of different *in vitro* assays. J. Drug Target. 10, 263–276.
[105] Lundquist, S., Renftel, M., Brillault, J., Fenart, L., Cecchelli, R. and Dehouck, M.P. (2002) Prediction of drug transport through the blood–brain barrier *in vivo*: a comparison between two *in vitro* cell models. Pharm. Res. 19, 976–981.
[106] Li, M.W., Mruk, D.D., Lee, W.M. and Cheng, C.Y. (2009) Connexin43 and plakophilin-2 as a protein complex that regulates blood–testis barrier dynamics. Proc. Natl. Acad. Sci. USA 106, 10213–10218.
[107] Nakase, T. and Naus, C.C. (2004) Gap junctions and neurological disorders of the central nervous system. Biochim. Biophys. Acta 1662, 149–158.
[108] Orellana, J.A. et al. (2009) Modulation of brain hemichannels and gap junction channels by pro-inflammatory agents and their possible role in neurodegeneration. Antioxid. Redox Signal. 11, 369–399.
[109] Rouach, N., Avignone, E., Meme, W., Koulakoff, A., Venance, L., Blomstrand, F. and Giaume, C. (2002) Gap junctions and connexin expression in the normal and pathological central nervous system. Biol. Cell 94, 457–475.
[110] De Bock, M., Kerrebrouck, M., Wang, N. and Leybaert, L. (2013) Neurological manifestations of oculodentodigital dysplasia: a Cx43 channelopathy of the central nervous system? Front. Pharmacol. 4, 120.
[111] Bruzzone, R., Haefliger, J.A., Gimlich, R.L. and Paul, D.L. (1993) Connexin40, a component of gap junctions in vascular endothelium, is restricted in its ability to interact with other connexins. Mol. Biol. Cell 4, 7–20.
[112] Gabriels, J.E. and Paul, D.L. (1998) Connexin43 is highly localized to sites of disturbed flow in rat aortic endothelium but connexin37 and connexin40 are more uniformly distributed. Circ. Res. 83, 636–643.

[113] Haefliger, J.A., Nicod, P. and Meda, P. (2004) Contribution of connexins to the function of the vascular wall. Cardiovasc. Res. 62, 345–356.
[114] Behringer, E.J. and Segal, S.S. (2012) Tuning electrical conduction along endothelial tubes of resistance arteries through Ca(2+)-activated K(+) channels. Circ. Res. 110, 1311–1321.
[115] Behringer, E.J. and Segal, S.S. (2012) Spreading the signal for vasodilatation: implications for skeletal muscle blood flow control and the effects of ageing. J. Physiol. 590, 6277–6284.
[116] de Wit, C. and Griffith, T.M. (2010) Connexins and gap junctions in the EDHF phenomenon and conducted vasomotor responses. Pflugers Arch. 459, 897–914.
[117] Haddock, R.E., Grayson, T.H., Brackenbury, T.D., Meaney, K.R., Neylon, C.B., Sandow, S.L. and Hill, C.E. (2006) Endothelial coordination of cerebral vasomotion via myoendothelial gap junctions containing connexins 37 and 40. Am. J. Physiol. Heart Circ. Physiol. 291, H2047–H2056.
[118] Schuster, A., Oishi, H., Beny, J.L., Stergiopulos, N. and Meister, J.J. (2001) Simultaneous arterial calcium dynamics and diameter measurements: application to myoendothelial communication. Am. J. Physiol. Heart Circ. Physiol. 280, H1088–H1096.
[119] Johnson, T.L. and Nerem, R.M. (2007) Endothelial connexin37, connexin40, and connexin43 respond uniquely to substrate and shear stress. Endothelium 14, 215–226.
[120] Toubas, J., Beck, S., Pageaud, A.L., Huby, A.C., Mael-Ainin, M., Dussaule, J.C., Chatziantoniou, C. and Chadjichristos, C.E. (2011) Alteration of connexin expression is an early signal for chronic kidney disease. Am. J. Physiol. Renal. Physiol. 301, F24–F32.
[121] Parthasarathi, K., Ichimura, H., Monma, E., Lindert, J., Quadri, S., Issekutz, A. and Bhattacharya, J. (2006) Connexin43 mediates spread of Ca2+-dependent proinflammatory responses in lung capillaries. J. Clin. Invest. 116, 2193–2200.
[122] Veliz, L.P., Gonzalez, F.G., Duling, B.R., Saez, J.C. and Boric, M.P. (2008) Functional role of gap junctions in cytokine-induced leukocyte adhesion to endothelium *in vivo*. Am. J. Physiol. Heart Circ. Physiol. 295, H1056–H1066.
[123] Little, T.L., Beyer, E.C. and Duling, B.R. (1995) Connexin43 and connexin40 gap junctional proteins are present in arteriolar smooth muscle and endothelium *in vivo*. Am. J. Physiol. 268, H729–H739.
[124] Traub, O., Hertlein, B., Kasper, M., Eckert, R., Krisciukaitis, A., Hulser, D. and Willecke, K. (1998) Characterization of the gap junction protein connexin37 in murine endothelium, respiratory epithelium, and after transfection in human HeLa cells. Eur. J. Cell Biol. 77, 313–322.
[125] Vis, J.C., Nicholson, L.F., Faull, R.L., Evans, W.H., Severs, N.J. and Green, C.R. (1998) Connexin expression in Huntington's diseased human brain. Cell Biol. Int. 22, 837–847.
[126] Larson, D.M., Haudenschild, C.C. and Beyer, E.C. (1990) Gap junction messenger RNA expression by vascular wall cells. Circ. Res. 66, 1074–1080.
[127] Theis, M. et al. (2001) Endothelium-specific replacement of the connexin43 coding region by a lacZ reporter gene. Genesis 29, 1–13.
[128] Wilson, A.C., Clemente, L., Liu, T., Bowen, R.L., Meethal, S.V. and Atwood, C.S. (2008) Reproductive hormones regulate the selective permeability of the blood–brain barrier. Biochim. Biophys. Acta 1782, 401–407.
[129] De Bock, M. et al. (2011) Connexin channels provide a target to manipulate brain endothelial calcium dynamics and blood–brain barrier permeability. J. Cereb. Blood Flow Metab. 31, 1942–1957.
[130] Lum, H., Jaffe, H.A., Schulz, I.T., Masood, A., RayChaudhury, A. and Green, R.D. (1999) Expression of PKA inhibitor (PKI) gene abolishes cAMP-mediated protection to endothelial barrier dysfunction. Am. J. Physiol. 277, C580–C588.
[131] Moriyama, Y., Takagi, N., Itokawa, C. and Tanonaka, K. (2013) Injection of neural progenitor cells attenuates decrease in level of connexin43 in brain capillaries after cerebral ischemia. Neurosci. Lett. 543, 152–156.
[132] Robertson, J., Lang, S., Lambert, P.A. and Martin, P.E. (2010) Peptidoglycan derived from Staphylococcus epidermidis induces connexin43 hemichannel activity with consequences on the innate immune response in endothelial cells. Biochem. J. 432, 133–143.
[133] De Bock, M. et al. (2013) Endothelial calcium dynamics, connexin channels and blood–brain barrier function. Prog. Neurobiol. 108, 1–20.
[134] De Bock, M., Wang, N., Bol, M., Decrock, E., Ponsaerts, R., Bultynck, G., Dupont, G. and Leybaert, L. (2012) Connexin43 hemichannels contribute to cytoplasmic Ca2+ oscillations by providing a bimodal Ca2+-dependent Ca2+ entry pathway. J. Biol. Chem. 287, 12250–12266.
[135] Anwar, Z., Albert, J.L., Gubby, S.E., Boyle, J.P., Roberts, J.A., Webb, T.E. and Boarder, M.R. (1999) Regulation of cyclic AMP by extracellular ATP in cultured brain capillary endothelial cells. Br. J. Pharmacol. 128, 465–471.
[136] Bintig, W. et al. (2012) Purine receptors and Ca(2+) signalling in the human blood–brain barrier endothelial cell line hCMEC/D3. Purinergic Signal. 8, 71–80.
[137] Simon, J., Vigne, P., Eklund, K.M., Michel, A.D., Carruthers, A.M., Humphrey, P.P., Frelin, C. and Barnard, E.A. (2001) Activity of adenosine diphosphates and triphosphates on a P2Y(T)-type receptor in brain capillary endothelial cells. Br. J. Pharmacol. 132, 173–182.
[138] Simon, J., Filippov, A.K., Goransson, S., Wong, Y.H., Frelin, C., Michel, A.D., Brown, D.A. and Barnard, E.A. (2002) Characterization and channel coupling of the P2Y(12) nucleotide receptor of brain capillary endothelial cells. J. Biol. Chem. 277, 31390–31400.
[139] De Bock, M. et al. (2012) Low extracellular Ca2+ conditions induce an increase in brain endothelial permeability that involves intercellular Ca2+ waves. Brain Res. 1487, 78–87.
[140] Leybaert, L. and Sanderson, M.J. (2012) Intercellular Ca(2+) waves: mechanisms and function. Physiol. Rev. 92, 1359–1392.
[141] Tien, T., Barrette, K.F., Chronopoulos, A. and Roy, S. (2013) Effects of high glucose-induced Cx43 downregulation on occludin and ZO-1 expression and tight junction barrier function in retinal endothelial cells. Invest. Ophthalmol. Vis. Sci. 54, 6518–6525.
[142] Manasson, J., Tien, T., Moore, C., Kumar, N.M. and Roy, S. (2013) High glucose-induced downregulation of connexin30.2 promotes retinal vascular lesions: implications for diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 54, 2361–2366.
[143] Sohl, G., Guldenagel, M., Traub, O. and Willecke, K. (2000) Connexin expression in the retina. Brain Res. Brain Res. Rev. 32, 138–145.
[144] Li, A.F. and Roy, S. (2009) High glucose-induced downregulation of connexin43 expression promotes apoptosis in microvascular endothelial cells. Invest. Ophthalmol. Vis. Sci. 50, 1400–1407.
[145] Bobbie, M.W., Roy, S., Trudeau, K., Munger, S.J. and Simon, A.M. (2010) Reduced connexin43 expression and its effect on the development of vascular lesions in retinas of diabetic mice. Invest. Ophthalmol. Vis. Sci. 51, 3758–3763.
[146] Andrade-Rozental, A.F., Rozental, R., Hopperstad, M.G., Wu, J.K., Vrionis, F.D. and Spray, D.C. (2000) Gap junctions: the "kiss of death" and the "kiss of life". Brain Res. Brain Res. Rev. 32, 308–315.
[147] Trudeau, K., Muto, T. and Roy, S. (2012) Downregulation of mitochondrial connexin43 by high glucose triggers mitochondrial shape change and cytochrome C release in retinal endothelial cells. Invest. Ophthalmol. Vis. Sci. 53, 6675–6681.
[148] Danesh-Meyer, H.V., Kerr, N.M., Zhang, J., Eady, E.K., O'Carroll, S.J., Nicholson, L.F., Johnson, C.S. and Green, C.R. (2012) Connexin43 mimetic peptide reduces vascular leak and retinal ganglion cell death following retinal ischaemia. Brain 135, 506–520.
[149] Danesh-Meyer, H.V., Huang, R., Nicholson, L.F. and Green, C.R. (2008) Connexin43 antisense oligodeoxynucleotide treatment down-regulates the inflammatory response in an *in vitro* interphase organotypic culture model of optic nerve ischaemia. J. Clin. Neurosci. 15, 1253–1263.
[150] Chew, S.S., Johnson, C.S., Green, C.R. and Danesh-Meyer, H.V. (2011) Response of retinal connexin43 to optic nerve injury. Invest. Ophthalmol. Vis. Sci. 52, 3620–3629.
[151] Cronin, M., Anderson, P.N., Cook, J.E., Green, C.R. and Becker, D.L. (2008) Blocking connexin43 expression reduces inflammation and improves functional recovery after spinal cord injury. Mol. Cell. Neurosci. 39, 152–160.
[152] Zahs, K.R., Kofuji, P., Meier, C. and Dermietzel, R. (2003) Connexin immunoreactivity in glial cells of the rat retina. J. Comp. Neurol. 455, 531–546.
[153] Zahs, K.R. and Ceelen, P.W. (2006) Gap junctional coupling and connexin immunoreactivity in rabbit retinal glia. Vis. Neurosci. 23, 1–10.
[154] Bringmann, A., Pannicke, T., Grosche, J., Francke, M., Wiedemann, P., Skatchkov, S.N., Osborne, N.N. and Reichenbach, A. (2006) Muller cells in the healthy and diseased retina. Prog. Retin. Eye Res. 25, 397–424.
[155] Ezan, P. et al. (2012) Deletion of astroglial connexins weakens the blood–brain barrier. J. Cereb. Blood Flow Metab. 32 (8), 1457–1467.
[156] Armulik, A., Abramsson, A. and Betsholtz, C. (2005) Endothelial/pericyte interactions. Circ. Res. 97, 512–523.
[157] Wakui, S. et al. (2006) Localization of Ang-1, -2, Tie-2, and VEGF expression at endothelial-pericyte interdigitation in rat angiogenesis. Lab. Invest. 86, 1172–1184.
[158] Winkler, E.A., Bell, R.D. and Zlokovic, B.V. (2011) Central nervous system pericytes in health and disease. Nat. Neurosci. 14, 1398–1405.
[159] Vigmond, E.J., Bardakjian, B.L., Thuneberg, L. and Huizinga, J.D. (2000) Intercellular coupling mediated by potassium accumulation in peg-and-socket junctions. IEEE Trans. Biomed. Eng. 47, 1576–1583.
[160] Caruso, R.A. et al. (2009) Ultrastructural descriptions of pericyte/endothelium peg-socket interdigitations in the microvasculature of human gastric carcinomas. Anticancer Res. 29, 449–453.
[161] Sohl, G. and Willecke, K. (2004) Gap junctions and the connexin protein family. Cardiovasc. Res. 62, 228–232.
[162] Li, A.F., Sato, T., Haimovici, R., Okamoto, T. and Roy, S. (2003) High glucose alters connexin43 expression and gap junction intercellular communication activity in retinal pericytes. Invest. Ophthalmol. Vis. Sci. 44, 5376–5382.
[163] Hirschi, K.K., Burt, J.M., Hirschi, K.D. and Dai, C. (2003) Gap junction communication mediates transforming growth factor-beta activation and endothelial-induced mural cell differentiation. Circ. Res. 93, 429–437.
[164] Borysova, L., Wray, S., Eisner, D.A. and Burdyga, T. (2013) How calcium signals in myocytes and pericytes are integrated across *in situ* microvascular networks and control microvascular tone. Cell Calcium 54, 163–174.
[165] Liddelow, S.A. et al. (2013) Mechanisms that determine the internal environment of the developing brain: a transcriptomic, functional and ultrastructural approach. PLoS ONE 8, e65629.
[166] Mercier, F. and Hatton, G.I. (2001) Connexin26 and basic fibroblast growth factor are expressed primarily in the subpial and subependymal layers in adult brain parenchyma: roles in stem cell proliferation and morphological plasticity? J. Comp. Neurol. 431, 88–104.
[167] Jovanova-Nesic, K., Koruga, D., Kojic, D., Kostic, V., Rakic, L. and Shoenfeld, Y. (2009) Choroid plexus connexin43 expression and gap junction flexibility are associated with clinical features of acute EAE. Ann. N. Y. Acad. Sci. 1173, 75–82.

[168] Bolborea, M. and Dale, N. (2013) Hypothalamic tanycytes: potential roles in the control of feeding and energy balance. Trends Neurosci. 36, 91–100.
[169] Frayling, C., Britton, R. and Dale, N. (2011) ATP-mediated glucosensing by hypothalamic tanycytes. J. Physiol. 589, 2275–2286.
[170] Giaume, C. (2010) Astroglial Wiring is Adding Complexity to Neuroglial Networking. Front. Neuroenergetics 2. pii: 129.
[171] Orellana, J.A. et al. (2012) Glucose increases intracellular free Ca(2+) in tanycytes via ATP released through connexin43 hemichannels. Glia 60, 53–68.
[172] Jarvis, C.R. and Andrew, R.D. (1988) Correlated electrophysiology and morphology of the ependyma in rat hypothalamus. J. Neurosci. 8, 3691–3702.
[173] Nabeshima, S., Reese, T.S., Landis, D.M. and Brightman, M.W. (1975) Junctions in the meninges and marginal glia. J. Comp. Neurol. 164, 127–169.
[174] Spray, D.C., Moreno, A.P., Kessler, J.A. and Dermietzel, R. (1991) Characterization of gap junctions between cultured leptomeningeal cells. Brain Res. 568, 1–14.
[175] Dermietzel, R., Traub, O., Hwang, T.K., Beyer, E., Bennett, M.V., Spray, D.C. and Willecke, K. (1989) Differential expression of three gap junction proteins in developing and mature brain tissues. Proc. Natl. Acad. Sci. USA 86, 10148–10152.
[176] Miragall, F., Hwang, T.K., Traub, O., Hertzberg, E.L. and Dermietzel, R. (1992) Expression of connexins in the developing olfactory system of the mouse. J. Comp. Neurol. 325, 359–378.
[177] Kunzelmann, P., Schroder, W., Traub, O., Steinhauser, C., Dermietzel, R. and Willecke, K. (1999) Late onset and increasing expression of the gap junction protein connexin30 in adult murine brain and long-term cultured astrocytes. Glia 25, 111–119.
[178] Nagy, J.I., Patel, D., Ochalski, P.A. and Stelmack, G.L. (1999) Connexin30 in rodent, cat and human brain: selective expression in gray matter astrocytes, co-localization with connexin43 at gap junctions and late developmental appearance. Neuroscience 88, 447–468.
[179] Malfait, M., Gomez, P., van Veen, T.A., Parys, J.B., De Smedt, H., Vereecke, J. and Himpens, B. (2001) Effects of hyperglycemia and protein kinase C on connexin43 expression in cultured rat retinal pigment epithelial cells. J. Membr. Biol. 181, 31–40.
[180] Calera, M.R., Topley, H.L., Liao, Y., Duling, B.R., Paul, D.L. and Goodenough, D.A. (2006) Connexin43 is required for production of the aqueous humor in the murine eye. J. Cell Sci. 119, 4510–4519.
[181] Udawatte, C., Qian, H., Mangini, N.J., Kennedy, B.G. and Ripps, H. (2008) Taurine suppresses the spread of cell death in electrically coupled RPE cells. Mol. Vis. 14, 1940–1950.
[182] Pocrnich, C.E. et al. (2012) The effect of connexin43 on the level of vascular endothelial growth factor in human retinal pigment epithelial cells. Graefes Arch. Clin. Exp. Ophthalmol. 250, 515–522.
[183] Pearson, R.A., Dale, N., Llaudet, E. and Mobbs, P. (2005) ATP released via gap junction hemichannels from the pigment epithelium regulates neural retinal progenitor proliferation. Neuron 46, 731–744.
[184] Brightman, M.W. and Reese, T.S. (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. J. Cell Biol. 40, 648–677.
[185] Roales-Bujan, R. et al. (2012) Astrocytes acquire morphological and functional characteristics of ependymal cells following disruption of ependyma in hydrocephalus. Acta Neuropathol. 124, 531–546.
[186] Li, X., Kong, H., Wu, W., Xiao, M., Sun, X. and Hu, G. (2009) Aquaporin-4 maintains ependymal integrity in adult mice. Neuroscience 162, 67–77.
[187] Marichal, N., Garcia, G., Radmilovich, M., Trujillo-Cenoz, O. and Russo, R.E. (2012) Spatial domains of progenitor-like cells and functional complexity of a stem cell niche in the neonatal rat spinal cord. Stem Cells 30, 2020–2031.
[188] Takahashi, M., Arai, Y., Kurosawa, H., Sueyoshi, N. and Shirai, S. (2003) Ependymal cell reactions in spinal cord segments after compression injury in adult rat. J. Neuropathol. Exp. Neurol. 62, 185–194.
[189] Wong, R.C., Pera, M.F. and Pebay, A. (2008) Role of gap junctions in embryonic and somatic stem cells. Stem Cell Rev. 4, 283–292.
[190] Corns, L.F., Deuchars, J. and Deuchars, S.A. (2013) GABAergic responses of mammalian ependymal cells in the central canal neurogenic niche of the postnatal spinal cord. Neurosci. Lett. 553, 57–62.
[191] Panchin, Y., Kelmanson, I., Matz, M., Lukyanov, K., Usman, N. and Lukyanov, S. (2000) A ubiquitous family of putative gap junction molecules. Curr. Biol. 10, R473–R474.
[192] Baranova, A. et al. (2004) The mammalian pannexin family is homologous to the invertebrate innexin gap junction proteins. Genomics 83, 706–716.
[193] Maslieieva, V. and Thompson, R.J. (2014) A critical role for pannexin-1 in activation of innate immune cells of the choroid plexus. Channels (Austin) 8. Epub ahead of print.