

## SPECIALIZED NEURAL BARRIERS

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**Abbreviations:** AD, Alzheimer's disease. APP, amyloid precursor protein. BLB, blood-labyrinth barrier. BNB, blood-nerve barrier. BRB, blood-retinal barrier. BSCB, blood-spinal cord barrier. CP, choroid plexus. CVO, circumventricular organ. ISF, interstitial fluid. MMP, matrix metalloproteinase. NDD, neurodegenerative disease. Pgp, P-glycoprotein. RPE, retinal pigment epithelium. TEER, transepithelial (or endothelial) electrical resistance, measure of tightness. TIMP, tissue inhibitor of MMP. TTR, transthyretin. SAH, subarachnoid hemorrhage. SAS, subarachnoid space. SCI, spinal cord injury. VEGF, vascular endothelial growth factor. VRS, Virchow-Robin space.

## **INTRODUCTION**

The importance of several specialized neural barriers is increasingly recognized. These barriers determine what will enter the compartments they serve, and account for many of the physiological properties of the specific neural environments. In addition, the recognition that disease may affect these barrier properties presents challenges in understanding pathology, and possibilities for new therapies.

In this report we consider the barrier sites between blood and neural tissue other than the endothelium of the brain parenchymal capillaries, the 'classical' site of the blood-brain barrier (BBB). Our list includes the blood-CSF barrier (choroid plexus epithelium); the meninges (pia and arachnoid) especially the blood-subarachnoid CSF barrier in the arachnoid; the blood-retinal barrier (retinal capillary endothelium; retinal pigment epithelium); the blood-nerve barrier (endoneurial capillary endothelium; perineurium); blood-labyrinth barriers (cochlea; vestibular system); and blood-spinal cord barrier. These barriers have been significantly less well studied than the BBB, but need to be considered together with the BBB as sites that influence the normal physiology of the neural microenvironment, that undergo specific patterns of developmental regulation, that show deterioration in aging, and express specific pathologies in several neurological disorders. Moreover, several of the neural barrier sites and mechanisms work together or in parallel, so a comprehensive overview needs to consider their individual function as well as their interactions.

### **1. CHOROID PLEXUS**

The choroid plexus (CP) is highly vascular, with leaky fenestrated capillaries in series with the barrier layer formed by the CP epithelium. The CP is the major site of production of CSF, it communicates via CSF with the interstitial fluid (ISF) of the brain, and it has a number of specific transport and secretory functions that contribute to growth and maintenance of neural tissue.

Both the CP and the CSF are involved in several pathologies, and CSF analysis can also give insights into conditions affecting the brain parenchyma and BBB.

#### A. Recent progress, choroid plexus barrier

- **Tight junctions.** The demonstration that although the CP TEER (typically 100-200 ohm cm<sup>2</sup>) is lower than that of the BBB (>1000 ohm.cm<sup>2</sup> *in situ*), the tight junctions are able to exclude small biotin-labeled EM tracers the size of sucrose and inulin.<sup>1</sup> Instead, some transcellular movement of these tracers has been observed in a small population of CP cells.<sup>2</sup>
- **Ion and water movements.** Identification of several ion transporters and ion channels responsible for CSF production, establishing in many cases their apical/basolateral CP distribution.<sup>3</sup> The demonstration that aquaporin-1 water channels on both membranes can act as an effective conduit for transcellular water flow.<sup>4-6</sup>
- **Organic molecule transport and detoxification.** Identification and localization of a number of uptake and efflux transporters and of highly expressed metabolic enzymes with overlapping specificities that mediate transport or metabolism of a range of organic molecules including drugs.<sup>7-15</sup> Transporters include (basolateral) Mrp1, Mrp4, Oatp2 and probably BCRP; and (apical) Oat3, Oatp3.<sup>13,16,17</sup> Apical Pept2 mediates transport of specific di- and tri-peptide.<sup>18</sup> Oat1 and Oatp1 are now not believed to be expressed as proteins.<sup>16</sup> P-glycoprotein (Pgp) is detected subapically, but there is no evidence for function.<sup>16</sup> Beta amyloid (A $\beta$ ) is cleared via a receptor-mediated process involving LRP2/megalin.<sup>19</sup>
- **Protein traffic.** The CSF protein levels are low in the adult, but endogenous proteins can transfer across the CP via a subpopulation of cells (~10%). The transport appears to be specific in the fetus; in the adult it is non-specific and overlaps with the population of biotin marker transfer cells. This contributes to evidence for cellular heterogeneity of the CP epithelium.<sup>20</sup>
- **Polypeptide secretory function.** Improved understanding of the secretory function of the CP, producing growth factors, cytokines and chemokines in the normal and injured brain, with actions on the CP via specific receptors, but also more widely within the CNS.<sup>21-23</sup> Better

understanding of the pathophysiological role of CP-derived polypeptides such as transthyretin (TTR) and gelsolin in normal aging and aging-associated neurodegenerative disease (NDD) e.g. Alzheimer's (AD).<sup>21,22,24,25</sup> The CP can also synthesize and secrete diffusible chemorepellents capable of providing guidance cues for growing axons.<sup>22</sup>

- **Pathogen adhesion and infection.** The BBB and CP are generally resistant to invasion by infectious organisms, but certain viruses, bacteria and parasites are able to enter the brain. Several studies have shown adhesion and penetration in isolated tissue and cell culture models, but for most infections, the major route of access is still unclear. In bacterial meningitis, *Streptococcus suis* can induce cell death in cultured porcine CP cells,<sup>26</sup> but the position in vivo is uncertain. *Neisseria meningitidis* does not appear to cross the CP, but rather enters via the BBB.<sup>27</sup> There is evidence for CP-mediated entry of HIV.<sup>28,29</sup>
- **Leukocyte trafficking.** Better appreciation of the role of CP and CSF pathways in leukocyte entry and distribution, based on new data on T-cell and neutrophil trafficking, and the demonstration of adhesion molecules ICAM-1, VCAM-1 and MadCam-1 in the CP.<sup>30-33</sup>
- **Matrix degradation.** The demonstration that inflammatory and infectious neurological disorders involve increased CP expression of MMPs 2 and 9 and TIMPs 2 and 3.<sup>34</sup>
- **CP grafting.** Demonstration of the therapeutic potential of grafted CP epithelial cells (free or encapsulated) in animal models of CNS injury and NDD,<sup>35,36</sup> and detection of stem cells/neural precursor cells in subependymal layers of the ventricular system.
- **CSF-ISF flow.** Significant progress in understanding the relations between CSF and ISF flow pathways,<sup>37</sup> with implications for volume transmission (chemical communication via extracellular fluid) especially of CP-derived peptides.<sup>21,22</sup>
- **CSF/ISF outflow pathways.** Increasing evidence that fluid drainage along blood vessels and cranial nerves (especially olfactory) to lymphatics may be the normal physiological outflow route in animals, with arachnoid granulations an auxiliary system to allow additional drainage in conditions of elevated intracranial pressure or when normal outflow routes are compromised.<sup>37,38</sup> The data is less secure in humans.

## **B. Barriers to progress for the choroid plexus**

These mainly stem from the lack of critical mass within the CP/CSF scientific community, limitations of model systems and access to human tissue, difficulty of designing experiments to unravel the complexity of the system in situ, and so far little application of the powerful techniques of proteomics and genomics.

## **C. Recommendations on studies needed for the choroid plexus barrier**

We need better information on CP function in health, pathology and aging, to provide insights leading to improved treatments and more effective drug delivery.

### **1. Basic understanding of CP in health and disease.** We need:

- New and improved in vitro models and methods to investigate basolateral proteins including transporters, leukocyte traffic and species differences. Tests with co-culture or exposure to CSF-borne agents, to reproduce a more fully 'induced' CP phenotype. More use of isolated CP tissue, to take advantage of differentiated CP with cell population and properties reflecting in vivo.
- Better designed studies to separate tight junctional (paracellular) and transcellular routes for molecular traffic, pathogenic organisms, and leukocytes, in aging and pathology. Influence of cytokines on tight junction stability. Reorganization of cytoskeleton, and role of extracellular matrix in pathology.
- More studies on the way CP activities including transport and secretion are affected by injury, NDD and aging, inflammation, atherosclerosis, stroke, hydrocephalus. What is the response of the CP, and what initiates this?
- Use of global analysis methods such as genomics and proteomics to pinpoint key proteins and pathways involved in human aging and disease, and to evaluate the adequacy of rodent models. Improved tools to explore barrier proteins.
- Greater attempts to link changes in expression levels of key molecules during development, aging and pathology, to changes in function, with well-defined quantitative measures.

- More detailed examination of the second messenger pathways involved in inflammation and CP modulation, to identify potential therapeutic targets.
- To explore differences in adhesion and access mechanisms for different classes and species of pathogen (viruses, bacteria, parasites such as malaria and larval helminthes), in parallel with studies on other barrier sites (BBB, arachnoid, CVOs) so that therapy can be directed early to the most vulnerable site(s).
- Greater understanding of CSF turnover dynamics in health and in specific disease states to understand the potential for CP/CSF delivery of drugs and removal of toxins and metabolites. Investigation of preferential pathways for ISF flow, including Virchow-Robin spaces (VRS), and flow mechanisms. Relation of CSF to ISF dynamics, leading to quantitative mathematical models for health and disease states.

**2. Manipulation of CP/CSF to improve health.** We need:

- Investigation of functional significance and role of CP in production of factors important for health and disease, e.g. cytokines and chemokines, growth factors such as IGF2, VEGF and TGF $\beta$ , as well as other proteins including APP, transferrin and TTR.
- To establish whether stimulants of multipotent cell differentiation in sub-ventricular layer can be delivered by CP/CSF.
- Further investigation of the ability of grafted CP epithelial cells to aid recovery in injury and NDD.
- Exploration of ways to manipulate CSF secretion rate, since stagnation correlates with NDD and excess with hydrocephalus. Excess is currently treated by insertion of a shunt device to shunt excess CSF to another absorption site, e.g., the peritoneal or pleural cavity, or directly into the blood stream. Suitable cases of obstructive hydrocephalus may be treated by III ventriculostomy. In the past surgical excision or electro-coagulation of CP tissue was carried out, or blunt, non-specific drug therapy, also affecting other secreting tissue (in e.g. kidney, eye) was given. Safe stimulation of CSF secretion could both lead to more effective drug delivery, and address a predisposing factor in NDD progression. In common with the

BBB, the key may lie in detecting specific proteins on the blood-facing membrane of the CP (here the basolateral membrane) to which treatments can be targeted.

- Focus on ISF/CSF outflow (reabsorption) pathways, and their characterisation, quantification, in normal function and in aging, pathology.

### **3. Drug delivery.** We need:

- More detailed genomic and proteomic profiling of the CP and CSF, followed up with functional studies, including the potential of the CP for secretion and transport, in both health and disease.
- Systematic analysis of the proteins expressed on the basolateral membranes, with particular focus on efflux transporters absent from BBB endothelium, since inhibition of these transporters could selectively enhance entry of lipid soluble therapeutics to CSF across CP while leaving BBB efflux intact.
- Thorough comparisons and validation of experimental models for expression of the range of receptors and transporters observed in vivo; transport function in intact tissue does not yet correlate well with the transporter expression so far documented.
- To identify ways to enhance (vesicular) secretory capacity of CP, or broaden substrate specificity.
- To determine the physiological significance of transport studies: which compounds can get from CSF to brain? Comprehensive study with a range of molecular chemistries including MW, lipophilicity, receptor specificity.
- Further case studies with drug compounds of the extent to which CSF analysis reflects brain ISF concentration, with the aim of establishing mechanistic information for CP vs BBB transfer, and general rules for ISF:CSF partition and compound turnover.

### **D. Recommendations on resources needed for choroid plexus barrier**

The CRISP database lists 23 currently funded NIH grants that include “choroid plexus” in the project summary or key words. In contrast, a search for “blood-brain barrier” pulls up over 7000

grants. Is research on choroid plexus underfunded? The key resource needed is motivated and well trained scientists, willing to extend into the field of CP/CSF pathology, and with a diversity of backgrounds and skills in order to bring new technologies to this area. Support is required for group infrastructure, personnel, consumables and access to specialized facilities.

We need:

- Access to human CP tissue and CSF banks, with samples from a range of pathologies where CP is implicated.
- Establishment of databases with information from genomics, proteomics and functional studies, to cover developmental stages, normal health, aging and pathologies.
- Resources specifically for development and validation of new models and methods – cellular imaging of isolated CP, isolated perfused CP from transgenic mouse models, induced and inverted CP models, CP models tight enough to study volume generation,.
- Resources to establish new methods to link work on cell and tissue models to human study, e.g. Synchrotron imaging and spectroscopy, higher resolution MRI.
- To develop mechanisms for independent assessment of evidence and replication of key findings, particularly where they are of clinical relevance.
- Development of systems that promote cooperation and collaboration, not competition: consortia of expert groups, better provision for collaboration between main geographical areas (Americas, Europe, Asia, Australasia) including joint funding initiatives, infrastructure and training grants, exchange programmes to build community networks and transfer technologies.
- Support for annual 2-day meetings to include postdocs and PhD students who do the work, to discuss results and coordinate activity.
- Support for Practical Courses or Workshops, to teach people how to carry out barrier and CP experiments; most needed for in vivo techniques, but also for consolidation of in vitro methods.

## 2. MENINGES

The meninges comprise three layers, the pia and arachnoid (making the leptomeninges) and the outer tough connective tissue of the dura. They all contain fibroblast-like cells, and show features of mesoderm, although both dura and leptomeninges may include derivatives from neural crest (ectoderm). The lamellated cell layers of the arachnoid extend trabeculae across the subarachnoid space (SAS), to meet the pia. The pial layer with its underlying basal lamina covers the end feet of the astrocytic glia that form the 'glia limitans' at the boundary of neural tissue. The arachnoid layer(s) bordering the dura are coupled by tight junctions, forming the meningeal barrier layer.

There have been relatively few studies of the arachnoid barrier, and its capacity to transport solutes is largely unexplored, although it can generate a significant (~40mV) potential difference, suggesting apical:basal differences in ion channels and transporters. The classical view of CSF drainage has been that it flows out via valve-like structures, outpouchings of the arachnoid (granulations) into dural venous sinuses, but alternative routes are gaining prominence (see choroid plexus section). Many studies of the leptomeninges do not distinguish between pia and arachnoid, although they have different morphology and functions. The pia-arachnoid is well vascularized, and while the microvessels show BBB properties, these vessels are both more leaky and more reactive than brain parenchymal vessels.

### A. Recent progress, meningeal barrier

- Pia-derived growth and chemoattractant factors attract neural precursor cells to a proliferative compartment beneath the pial surface.
- Meninges around the hindbrain provide a rich source of retinoic acid in neural development and pattern organization.<sup>39</sup>

- The pial membranes form a substrate for migration of neural precursor cells important in cerebral cortical development, and regulate their dispersion pattern via secreted chemokines.<sup>40</sup>
- During human fetal development, the first intracerebral microglia are seen at 5-6 gestational weeks (gw) close to the meninges and choroid plexus, and develop associations with parenchymal blood vessels later, at 10-12 gw.<sup>41</sup>
- Adenosine deaminase (ADA) is highly expressed in the barrier but not trabecular layer of the arachnoid, playing an important role in metabolism of adenosine (sleep factor) in the SAS, and regulating non-REM sleep.<sup>42</sup>
- Following injury to the brain, leptomeningeal fibroblasts invade the lesion core, contribute to the forming glial scar, and inhibit neurite outgrowth by secretion of semaphorins.<sup>43</sup>
- Several pathologies including subarachnoid hemorrhage (SAH) and bacterial infections involve inflammatory lesions of the pia/arachnoid and its vessels, but in the case of bacterial meningitis (*Neisseria meningitides*), the initial infection may be via the brain parenchymal vessels; it is not clear how invasion across the BBB results in inflammation of the meninges and not the parenchyma.<sup>27</sup>
- In infection with a larval helminth parasite, the development of extravasation of leukocytes and plasma proteins was most severe in the pial vessels, least in parenchymal vessels, confirming regional heterogeneity of the vascular bed.<sup>44</sup>

## **B. Barriers to progress for the meningeal barrier**

Few scientists involved in neural barrier research work on the meninges. We lack good experimental models of the arachnoid barrier layer and of the pial layer. Most studies on the pia have focused on its role in regulating neural development and migration, with few studies in adult, aging or pathology. Studies of SAH have mainly concentrated on the vasospasm, toxicity and inflammatory aspects, not on barrier effects. CNS pathologies frequently involve several barrier sites (BBB, choroid plexus, meninges) but are rarely considered together. Few clinicians working on infections or parasitic invasion of the meninges interact with barrier scientists.

### **C. Recommendations on studies needed for the meningeal barrier**

- Develop better models, of the meningeal cell layers singly and together, and mimicking pathologies.
- More coordinated attack on different barrier sites in particular pathologies, especially meningitis, SAH, parasitic infections.
- Application of modern technologies, as for BBB and choroid plexus.

### **D. Recommendations on resources needed for the meningeal barrier**

- Workshops to bring together clinicians and basic scientists in this field
- Funding initiatives to develop specific models identified as necessary for progress in understanding.
- Focused research programmes on the main clinical conditions affecting meningeal function.

## **3. BLOOD-RETINAL BARRIER**

The retina is formed by an outpouching and invagination of the neural tube, with both the neuronal layer (receptors, retinal neurons) and the retinal pigment epithelium (RPE) being derived from the neuroepithelium. In the adult, the photoreceptors and RPE, choroid plexus and ependyma preserve many features of the primordial ependymoglial layer. The RPE and receptor layer receive nutrients via the highly vascular and leaky choriocapillaris, while the inner retina (neural layers) is supplied by the central retinal artery. The inner blood-retinal barrier (BRB) is formed by the endothelial cells of the retinal capillaries, with properties similar to those of the BBB, while the outer BRB is formed by the RPE; overall the BRB is more leaky than the BBB, allowing greater penetration of hydrophilic compounds. The retinal microvessels have an approximately four times higher density of pericytes compared to brain microvessels, but the significance of this is unclear. The RPE contributes to transport of amino acids and fatty acids to

the outer retina, and is important in visual function by clearing debris of shed receptor outer segments, and re-cycling *retinal*.

### **Pathologies of BRB breakdown**

**Capillary endothelium:** Retinopathy – diabetic & hypertensive, retinopathy of prematurity, blood diseases, retinal vascular occlusion, trauma, surgery, tumors – retinoblastoma & hemangioblastoma, posterior uveitis.

**RPE:** Age-related macular degeneration, choroidal ischaemia, trauma, surgery, posterior uveitis.

### **A. Recent progress on blood-retinal barrier**

- Most significant development: recognition of the role of vascular endothelial growth factor (VEGF) in the pathogenesis of vasoproliferative ocular disorders such as diabetic retinopathy and choroidal neovascularization ('wet' age-related maculopathy).<sup>45</sup> This has led to the development of anti-VEGF agents effective in treating these conditions.
- Demonstration that both inner and outer BRB are subject to inductive influences; from astrocytes and Muller cells on endothelium, and retinal tissue on RPE, in development and maintenance of the barriers.<sup>46</sup> Some progress in identifying the inductive signals.
- RPE: Better understanding of solute transporters; and apical:basal ion channels and transporters regulating fluid transport and ionic composition of the subretinal space. Detection of MRP, and functional Pgp, the latter both apical and basolateral; the role of the apical Pgp is uncertain, but may be related to ion channel regulation or lipid transport.<sup>47</sup>
- Better understanding of the permeability of different layers of the eye (sclera, conjunctiva, inner and outer BRB), with impact on drug delivery. Intravitreal injection and implantation is a relatively safe way of by-passing eye barriers, making possible retinal treatment with agents and particulates (e.g. nanoparticles) difficult to deliver to brain.<sup>48</sup>
- Demonstration of importance of pericytes in controlling blood flow in capillaries.<sup>49</sup>

### **B. Barriers to progress for the blood-retinal barrier**

Eye barriers remain a minority interest. Using key words 'blood retinal barrier' yielded 285 Medline references post 2000, compared to 6,500 references for 'blood brain barrier'. In the intact eye, it is difficult to separate effects of inner and outer BRB, and studies on isolated retina are difficult. There is a lack of good in vitro models, including cell lines. We lack suitable animal models for many eye pathologies. We still have poor understanding of factors determining and limiting drug delivery to the retina, less information than for BBB. Recent advances in genomics and proteomics have not yet been applied to the BRB.

#### **C. Recommendations on studies needed for the blood-retinal barrier**

- Ocular drug delivery remains a priority area. Parallel developments in understanding the pathogenesis of ocular disease have revealed several therapeutic possibilities. Need to enhance mechanistic understanding of permeability and transport for effective therapeutic discovery and development.
- Take more advantage of the ability to image the retina, and accessibility by injection for targeting to inner and outer retina.
- Develop better models, methods.
- Parallel programmes combining basic and clinical approaches.
- Introduce new technologies: engineering, nanoparticles, MRI imaging, genomics and proteomics.

#### **D. Recommendations on resources needed for the blood-retinal barrier.**

- Funding to form consortia of groups, to build critical mass in this community.
- Use the powerful tools developed for imaging human retina (e.g. laser scanning ophthalmoscope), to tackle questions relevant to BRB and BBB: leukocyte trafficking, modulation of permeability, changes in aging and pathology.

### **4. BLOOD-NERVE BARRIER**

The blood-nerve barrier (BNB) comprises two barrier sites – the endothelium of endoneurial capillaries, and the perineurium (sheath) surrounding the bundles (fascicles) of axons. Both are tight-junction coupled layers: endothelial (capillaries) vs. epithelial (perineurium). The capillary endothelium shows many (though not all) of the features reported for brain capillaries, and most evidence indicates it is somewhat more leaky than the BBB. The perineurium is a continuation of the arachnoid barrier layer of the CNS, and as with the arachnoid, its embryological origin is uncertain; it shows some features of ectodermal (neural crest) as well as a mesodermal (fibroblastic) phenotype. Both barriers can be disrupted, especially following surgery or trauma, but also in autoimmune disorders (e.g. Guillain-Barre syndrome, GBS), inflammation (diabetic neuropathy), and chronic demyelinating polyradiculoneuropathy (CIDP); disruption is accompanied by intraneural edema. Both barriers can repair following injury. Many studies use the term BNB without specifying which barrier layer is being investigated.

#### **A. Recent progress on the blood-nerve barrier**

- Identification of *desert hedgehog* (Dhh) a signalling molecule from Schwann cells, and factors from pericytes, in barrier induction in perineurium and endothelium respectively.<sup>50</sup>
- Presence of Pgp in BNB – from immunocytochemical and pharmacokinetic evidence.
- BNB in rat develops in postnatal days P13-P16, based on EM and dye studies.<sup>51</sup>  
Corresponding tight junctional development in human embryo.<sup>52</sup>
- Thrombomodulin expression is low in BBB and perineurial vessels, moderate in endoneurial and epineurial vessels, suggesting inverse correlation with barrier function.<sup>53</sup>
- Opening of BNB following crush, in inflammation, and in Wallerian degeneration. Mediated by MMPs 7,9,12 from Schwann cells, endothelium and macrophages; a more vigorous response than to inflammatory agents at the BBB.<sup>54,55</sup> iNOS and TNF $\alpha$  involved.
- Perineurium of sciatic nerve resistant to modulation by inflammatory mediators, as demonstrated by correlated EM tracer and electrophysiological study.<sup>56</sup>

- In dorsal root ganglion (DRG), perineurium moderately tight, but some vessels are leaky: regional differences between cell body region (leaky) and nerve fibre region (tighter).<sup>57</sup> Tightness correlated with presence of Claudin-5 and occludin.<sup>58</sup> Peripheral myelin protein 22 (PMP22) is present at tight junctions in barrier sites, a useful early marker of barrier maturation.<sup>59</sup>
- In some sites, perineurium is more leaky, vulnerable – e.g. oculomotor nerve head emerging from brain stem.
- Grafting and repair of peripheral nerve – avoid adhesions impairing perineurium recovery by injecting alginate sol,<sup>60</sup> or using a biocompatible cuff around the nerve. End-to-side nerve grafting is more effective when a window is cut in the perineurium to encourage collateral sprouting and regeneration.<sup>61</sup>
- MRI with gadolinium – sufficient resolution to follow changes in intraneural edema.

#### **B. Barriers to progress for the blood-nerve barrier**

The scientific community working on nerve barriers is small, although many clinicians and basic scientists work on peripheral nerve repair. The small size of nerve tissue samples (from animals, human biopsies) makes resolution difficult. Few studies quantify barrier permeability; often difficult to separate perineurial and capillary components. Cell culture models of nerve endothelial cells are available, but not of perineurium. Relatively few techniques are applied for examining protein expression, permeability. There is insufficient attention to time-course of development and repair of lesions, and almost no proteomics or genomics.

#### **C. Recommendations on studies needed for the blood-nerve barrier**

- Examining species differences e.g. rodents, pigs (often used to develop surgical procedures for humans), humans.
- More comprehensive structure-function studies, designed for clearer separation of perineurial and endothelial components.

- Careful monitoring of time course of development and recovery of barrier breakdown following lesion.
- Extend electrophysiological and isotopic methods as well as new vital dye imaging methods to quantify changes in BNB permeability.
- Determine sites and mechanisms for entry of inflammatory cells.
- Make more use of temporary opening of BNB in pathology to deliver drugs specifically.
- Use of proteomics, genomics.

#### **D. Recommendations on resources needed for the blood-nerve barrier**

- Teams with multiple expertise working together – clinical (surgical, neurological, gross electrophysiological) and basic (immunocytochemical, microelectrophysiological, permeability assays) - to generate new information, as quantitatively as possible.
- Better definition of the nature of the disturbance in pathologies, in order to target therapy.
- Involvement of biomaterials chemists/engineers in generating the best matrix/substrates to encourage nerve regrowth and BNB repair following trauma or surgery.

### **5. BLOOD-LABYRINTH BARRIERS (BLB): cochlea, utricle, saccule, semi-circular canals**

#### **A. Recent progress on the blood-labyrinth barrier**

The concept of a BLB arose from the observation of markedly different chemical compositions for blood and inner ear fluids.<sup>62</sup> The BLB includes a blood-endolymph barrier and a blood-perilymph barrier. Anatomically, the BLB consists of tight junctions between specialized epithelial cells in the walls of the labyrinth spaces, both vestibular and cochlear.<sup>63</sup> Capillaries running close to sensory (hair) cells and axons have tight junction coupled endothelium with BBB characteristics. BLB function is strongly influenced by physiologic factors such as active and passive membrane functions, ion channels, specific transport systems, blood flow, hypoxia, inflammation, auto-immunity, cytokines, free radicals, stress hormones, trauma, noise exposure, toxicity and other

factors. The BLB affects cochlear and vestibular pharmacokinetics of many compounds, delaying entry of some and restricting egress of others.

The BLB is important in regulation of Na<sup>+</sup> and K<sup>+</sup> ions as well as the passage of other molecules partially depending on molecular weight.<sup>62</sup> Cochlear recycling of K<sup>+</sup> is important for hair cell function so any process that disrupts flow of K<sup>+</sup> ions may adversely affect auditory or vestibular function.<sup>64</sup> Some gene disorders<sup>65</sup> and pathologic conditions (inflammation, Meniere's disease, presbycusis for example) may involve alterations of the BLB and ion homeostasis.<sup>66</sup>

Compared to other specialized neural barriers, knowledge about the BLB is at an early stage. It is likely that important knowledge can be gained by understanding the differences between the BLB and other blood-neural barriers.

#### **B. Barriers to progress for the blood-labyrinth barrier**

Funding shortages are the major limitations to progress in BLB knowledge. The BLB has been described relatively recently and its importance is only slowly being recognized, but it remains a minority interest ('blood labyrinth barrier' gave only 15 Medline references post 2000). There is declining interest in basic science in clinical educational programs resulting in a paucity of investigators for translational research. Several clinical research investigations have been initiated without much consideration of the role of the BLB. Physical limitations of the ear make study of the inner ear fluids challenging. The inner ear is encased in bone which complicates many current instrumentation techniques. Delivery of drugs to the inner ear via the round window route is becoming popular in spite of a lack of understanding of the permeability of the round window in health and disease. Differentiation between BLB properties and other inner ear physiology is unclear.

#### **C. Recommendations on studies needed for the blood-labyrinth barrier**

- Development of animal models, and new methods for investigating the intact cochlea. Studies of the normal function and repair of the BLB and how physiologic processes affect permeability of the BLB for different compounds. Knowledge of the effects of trauma on the BLB including noise, and the effects of aging.
- Understanding the BLB in health, and changes in different pathologies. The roles of reactive oxygen and nitrogen species, cytokines, trauma and hair cell regeneration. The effects of size and molecular weight, polarization and other chemical properties on the permeability of the BLB to different chemical species.
- Implications for BLB when new drugs are administered to the inner ear in clinical situations (e.g. corticosteroids and aminoglycosides).
- Use of nanotechnology techniques in cochlear physiology.
- Development of nanoliter scale analytic resources. Creation of techniques for measuring drug concentrations in perilymph and endolymph to permit study of the pharmacokinetics of drugs and protective agents in the inner ear.
- Opportunities to discuss similarities and differences between the BLB and other barriers.

#### **D. Recommendations on resources needed for the blood-labyrinth barrier**

- Increased funding for neural barriers research by both private and public agencies.
- Greater interest in the pharmacokinetics of the inner ear needs to be generated. More investigators and more cross-collaboration between individuals interested in Blood–Neural barriers.
- Greater input from other areas of cell biology and physiology as these apply to the BLB. Feedback from human clinical experience to provide insight into cellular mechanisms in the BLB.

## **6. THE BLOOD-SPINAL CORD BARRIER (BSCB)**

Compared with the BBB, the BSCB has several distinctive features. Like the BBB it is an endothelial cell barrier reinforced by astrocytes, pericytes, and extracellular matrix, but the spinal cord has no choroid plexus. The transition to the peripheral system is related to nerve root entry zones, rather than circumventricular organs. The general permeability is higher than that of the BBB. The regional blood supply has a different array of segmental distribution and anastomoses than that in the brain, leading to different distribution of watershed zones and white matter vulnerability to ischemia. CSF contacts the spinal cord at its surface (spinal subarachnoid space) and interior (central canal); leptomeninges and ependyma respectively form the interface layers, but without significant barrier function under normal conditions.

#### **A. Recent progress in blood-spinal cord barrier**

- Better understanding of the pathophysiology and experimental therapeutics involved in spinal cord injury (SCI). Alterations of spinal cord blood flow and tissue perfusion, mechanisms of secondary injury, and the biphasic opening of the BSCB illustrate how better understanding of the BSCB will facilitate designs for optimal drug delivery.<sup>67</sup>
- The upregulation of selective transport systems for proinflammatory cytokines across the BSCB in disease processes indicates that the barrier function is not static, but rather is modulated by ongoing tissue remodeling processes. The active regulatory functions of the BSCB in turn influence spinal cord regeneration and functional recovery.
- In addition to mechanical trauma shown in different models of SCI (contusion, compression, transection, hemisection), progress has been made in addressing other etiologies, including tumor compression, ischemia, irradiation, inflammation induced by pathogens or chemicals, and autoimmune diseases.
- Experimental autoimmune encephalomyelitis (EAE) provides a good model for investigation of the ascending progression of demyelination, inflammation, and the intricate relationship between tissue damage and increased BSCB permeability.<sup>68</sup>
- The methodology to examine BSCB is now more versatile. Pharmacokinetic studies have been performed with small tracers (sucrose, inulin, mannitol, aminoisobutyric acid) and larger tracers

(albumin, horseradish peroxidase, immunoglobulin, luciferase) that illustrate dynamic changes in disease processes.<sup>69,70</sup> Histological examination shows distinctive differences in BSCB between white and gray matter, and among epicenter, caudal, and rostral regions of the spinal cord. Magnetic resonance imaging both in vivo and post mortem provide high resolution information on hemorrhage, edema, and BSCB breakdown.

- Comparison of primary microvessel endothelial cells derived from the brain and spinal cord complement in vivo studies to demonstrate lower levels of expression of certain tight junction proteins and higher level of transferrin receptor.<sup>71</sup>

### **B. Barriers to progress for the blood-spinal cord barrier**

The BSCB is an even smaller community than many other groups studying specialized barriers. Despite the rush of many laboratory groups to identify cures for SCI, studies on BSCB require relatively specialized techniques and thorough understanding of spinal cord anatomy. There has been limited funding support for BSCB research.

### **C. Recommendations on studies needed for the blood-spinal cord barrier**

- BSCB researchers need to identify the milestones to be accomplished, and the general scientific community needs to recognize the implications coming from BSCB research.
- Developmental studies to determine how the peripheral nervous system and circulatory factors modulate BSCB permeability.
- Identification of the mechanisms of migration and penetration of inflammatory cells and large molecules across the BSCB.
- Identify how the BSCB is affected by individual cellular components and extracellular matrix in temporal and spatial patterns of spinal cord regeneration.
- Determine how stem cells, nerve grafts, and other therapeutic reagents modify BSCB function.
- Target the BSCB to enhance drug delivery to treat autoimmune, injurious, inflammatory, and ischemic disorders in the spinal cord.
- Examine the communication between BSCB and BBB and how this improves neuroplasticity.

- Investigate dynamics of ISF/CSF flow in spinal cord in relation to brain, and effects on drug delivery, hydrocephalus. Effects of trauma/surgery on fluid flow, and relevance for delivery of growth factors to potential repair zones.

#### **D. Recommendations on resources needed for the blood-spinal cord barrier**

- Facilities for high resolution in-vivo imaging to follow the dynamic changes of the BSCB in disease processes.
- Communication between neurosurgeons and laboratory researchers for establishment of translational research.
- Better reception of BSCB proposals by the BBB and SCI communities and their funding resources.
- Mechanisms to attract more young scientists with patience and dedication for the challenging tasks of BSCB research.

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#### **SUMMARY**

This survey of specialized neural barriers of clinical relevance shows many common themes – the nature of the cell layers responsible for the barrier, their permeability and transport function, the things that can go wrong in pathology, and the most promising therapeutic avenues. However, it is clear that each of these barrier sites also shows unique features that not only arise by a tissue-specific developmentally regulated process, but also function somewhat differently in normal physiology, and show particular vulnerabilities in pathology. In many cases the differences highlight the advantages or disadvantages of the particular tissue for experimental examination, e.g. it is easier to look into the eye than the brain non-invasively; similarly eye drug delivery is more straightforward. The differences also demonstrate the distinct ways in which particular tissues deal with similar physiological problems. While examination of each barrier site can give

valuable insights into development, physiology and pathology, many of them work together in such a coordinated way that we need to have systems and models in place to explore this complexity. This implies much better integration between groups, and greater emphasis on optimizing *in vivo* preparations and models capable of addressing questions related to coordinated barrier function. For all these specialized barriers similar strategic issues arise - the need to develop critical mass within the specific scientific community and to link with other groupings, to identify key clinical conditions requiring understanding and treatments, and to apply state-of-the-art technologies to gain new and better information to underpin translational developments.

## **CONCLUSION**

The most important issue for specialized barriers is **understanding the similarities and differences among neural barriers**. Similarities will help in understanding the generality of particular features and findings, while differences will highlight the alternative mechanisms for achieving neural protection available within the barrier repertoire, and their differential disturbance in pathology. The hope and expectation is that the newly formed IBBS will act as an excellent forum for presentation of information and ideas, for debate, and for strategy development. This will facilitate not only the integration needed between fields and communities, but also the identification and application of the steps required to take key scientific findings through the translational process to sustain healthy aging and treat neural pathologies.

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## Figure Legends:

### Figure 1. Location of barrier sites in the CNS.

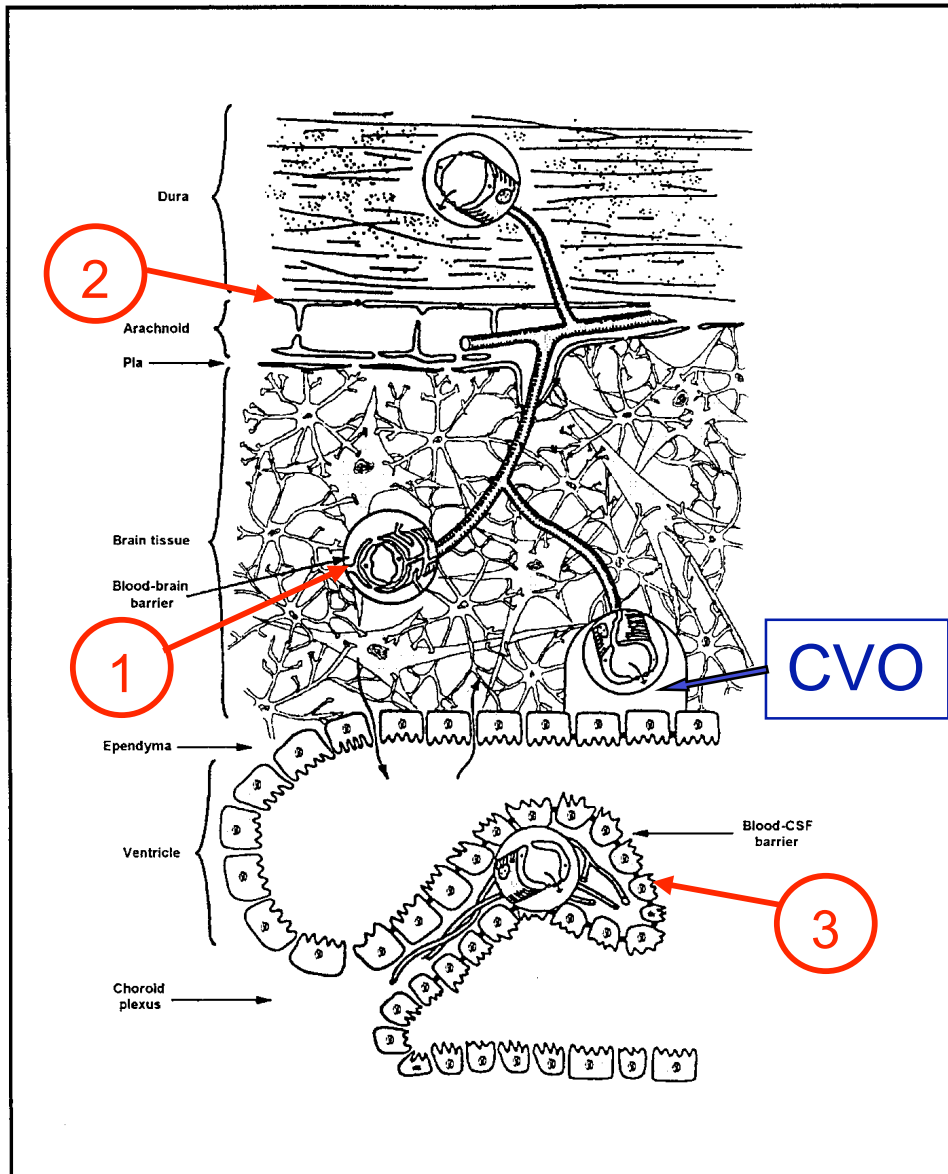
Barriers are present at three main sites: 1) the brain endothelium forming the blood-brain barrier (BBB), 2) the arachnoid epithelium forming the middle layer of the meninges, and 3) the choroid plexus epithelium which secretes cerebrospinal fluid (CSF). In each site, the physical barrier is caused by tight junctions that reduce the permeability of the paracellular (intercellular cleft) pathway. In circumventricular organs (CVO), containing neurons specialised 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. (Based on Segal & Zlokovic, 1990, modified by A Reichel; from Abbott, 2004)<sup>37</sup>. The other specialized endothelial barriers covered in this report (e.g. in eye, nerve, spinal cord) are similar to the brain endothelial barrier, while the other epithelial barriers (in eye, nerve, labyrinth) show more regional specializations.

### Figure 2: Summary of events at the blood-brain barrier in neurological disorders.

Leukocytes (L) attach to activated brain endothelial cells (EC) through cell adhesion molecules (CAMs). Activation of the brain ECs and induction of intracellular signaling pathways through CAMs or inflammatory mediators (IMs) like reactive oxygen species, cytokines, chemokines and matrix metalloproteinases, facilitate leukocyte migration and extravasation and leakage of serum proteins through an impaired blood-brain barrier. After passage across the brain endothelial basement membrane (EBM), inflammatory cells accumulate in the perivascular space (PVS) which is enclosed by the parenchymal basement membrane (PBM). These pathological processes also involve other central nervous system cells such as pericytes (P), perivascular macrophages (PVM), and microglial cells ( $\mu$ G) which secrete IMs, and astrocytes which lose their protective endfeet projections (A; indicated on the left). Processes in endothelia of other neural barrier sites such as the retina, spinal cord and meninges, although less investigated,

show similar patterns of activation in pathology. Illustration by permission of Elga de Vries.<sup>72</sup>

Figure 1.



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Figure 2.

