Choroid Plexus Blood-CSF Barrier: Major Player in Brain Disease Modeling and Neuromedicine
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ABSTRACT

The choroid plexus (CP) of the blood-cerebrospinal fluid barrier (BCSFB) impacts CSF homeostasis, brain diseases and neuromedical translation. CP executes neuroendocrine, excretory, and neuroimmune actions. BCSFB diversely manages brain-spinal cord fluid environments, giving rise to a wide pathophysiology spectrum. Newly-discovered choroidal phenomena include integration of circadian clock signals, immune interaction with gut microbiota, and expression of receptors to taste CSF composition. BCSFB tight junctions and transcellular mechanisms differ from blood-brain barrier (BBB) counterparts, variably regulating pathogen and leukocyte access to the CSF-brain nexus. This review highlights microbial agents, substrates and autoantigens using CP epithelial membranes to penetrate CSF and periventricular regions. Lipopolysaccharide (LPS) is analyzed as a barrier-damaging agent and neuroinflammation promoter. Transducing LPS- and toll-like receptor activity produce CP-CSF cytokines in sickness behavior and virulent sepsis-associated encephalopathy. Agents/systems that counter oxidative activity such as matrix metalloproteinase 8 inhibitors and Nrf2 activators (bile acids and isothiocyanates) show promise as neural and CP protectants. One review theme emphasizes CP’s preponderant role in initiating central diseases, and their remediation. In view of BCSFB permeativity alterations and epithelial transformation, we discuss: systemic lupus erythematosus, N-methyl D-aspartate-associated autoencephalitis, helicobacter disruption of BCSFB, toll-like receptor 2 stimulation in CP (neuroinflammation), the CP gateway for trypanosomes, and APOE-linked cholesterol transport into CSF. Another section treats concurrent involvement of BCSFB-BBB alterations in helminthic meningitis, forebrain ischemia, acute hyperthermia, leptin resistance/obesity, diabetes mellitus, and Alzheimer-type neurodegeneration. Barrier impairment is analyzed by injury type, time course, therapeutic strategies, and translational neuromedicine principles. The restorative power of BCSFB-transported growth factors, hormones and medicinal agents is emphasized for strengthening CP-CSF homeostatic mechanisms in seizures, stroke and Parkinsonism. A worthy therapeutic aim is to attenuate CNS disorders triggered by BCSFB malfunction, using CSF-delivered therapeutic agents for promoting neural viability.

Introduction: Role of Barrier-Interfaces in Maintaining Brain Viability

Neuronal networks require stable extracellular fluid (ECF). Neurotransmission and electrical signaling depend on ECF of specialized composition. Transport/barrier/secretory systems at the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) primarily regulate ECF. Choroid plexus (CP) is the main locus of the BCSFB, but circumventricular organs/meninges also contribute. There has been a tendency to underestimate BCSFB importance in favor of BBB. The two
systems, however, are usually coordinated. Both barriers contain elements of the innate immune system and secrete messages (cytokine signals) into brain and CSF.

The BBB and BCSFB provide countless substrates via regulated carrier transport to the CNS (Fig. 1). Both are ‘guardians’, acting as barriers to thwart diffusion of potentially injurious molecules. More properly, CNS barrier systems function as ‘barrier-interfaces’ because of great solute exchange along with their barrier (impermeability) function. Thus, the dual BBB and BCSFB interfaces display dynamic transport physiology as a cardinal feature.

In mediating ECF homeostasis, CNS transport interfaces use intricate mechanisms. Tight junction components manifest as heterogeneous endothelial and epithelial membranes. Cerebral capillaries utilize a distinct array of transporters that deliver mainly glucose, amino acids and free fatty acids (FFA) to nearby neurons. Choroidal epithelial cells, innate immune response, and neuroinflammation.

Brain microvessels chiefly translocate amino and fatty acids as well as glucose into parenchyma. CP specializes in secreting numerous trophic/stabilizing proteins, e.g., brain-derived neurotrophic factor, transthyretin, hormones and micronutrient vitamins. Choroidal and cerebral endothelial secretions physically combine, especially near brain parenchyma, to form the ECF milieu for neurons.

CSF movement thoroughly mixes all fluids in the CNS. CSF contacts virtually every barrier interface. CP primarily generates CSF that flows down the ventricles to the basal cisterns in the subarachnoid space (SAS); from there, CSF distributes widely to far reaches of the brain/cord (Fig. 2). Multi-regional transport activities and CSF flow create an ECF blend (‘hybrid’) of BBB and CP secretions. Fluid blending is facilitated by the paravascular system that starts as SAS CSF entering the superficial cortex at the Virchow-Robin spaces (Fig. 2). Overall, then, the driving force that propels CSF forward into the ventricular, subarachnoid and paravascular systems originates largely at the briskly-perfused and secreting CP villi.

Each CP villus contains a rich, inner vascular core, and adjacent interstitium. Both are surrounded by a ring of epithelial cells in adults (Fig. 3). The rapidly-metabolizing epithelium is highly equipped with organelles (Fig. 4). Abundant endoplasmic reticulum, golgi apparatus and mitochondria enable profuse synthesis of proteins and peptides (Tables 1 and 2). Upon secretion into CSF, these choroidally-derived proteins/peptides flow through the ventriculo-SAS to neuronal and glial targets. Targeted regions are both proximate to and distant from CP tissues. Upregulated BCSFB secretions into CSF-ECF abet healing of damaged neurons.

**Choroid Plexus and BBB Disruption: Extracellular Dyshomeostasis Places Neurons at Risk**

The first sequential casualty of barrier injuries is the nearby ECF composition. Distorted ECF biochemistry disables neuronal/synaptic functions. This disablement is proportional to transporter breakdown and induced leakiness. Before discussing specific disorders/diseases that destabilize the barriers, we lay out the general features of i) barrier physiology: transporters, solute gradients across barriers, and mitochondrial energetics, and ii) barrier pathophysiology: cytokine disruption of barrier cells, innate immune response, and neuroinflammation. The immediately-following paragraphs overview general physiologic principles, together with models and experimental tools associated with barrier studies, as a prelude to the later treatment of specific barrier injuries/pathologies.

**Barrier transport/energetics phenomena**

The complexity of solute transporters and channels in barrier cells, and their regulation by receptor and enzymatic activities, cannot be overstated. It is beyond the present scope to detail the myriad of transporters at the BCSFB...
Table 1. Newly appreciated and established functions/actions at the choroidal blood-cerebrospinal fluid interface

- Integrator of circadian clock periodicity signals, generated in the choroidal syncytium and conveyed to the suprachiasmatic pacemaker in the hypothalamus
- Source of stem cells and progenitor elements, putatively for the reconstitution of CP epithelium and periventricular regions, following injuries such as ischemia and trauma
- Relay station for plasma hormone signals that, once secreted into the ventricles, reach the hypothalamus for hormonal regulatory phenomena, e.g., feedback inhibition
- Gateway through which microbes (e.g., bacteria, viruses and trypanosomes) from intestine (and other regions) invade the blood, and then distribute to and injure the choroid plexus-CSF system
- Interface for immunologic molecules and cells to access the epithelium, thereby generating cytokine signals and activating leukocyte transfer to the CSF-periventricular nexus
- Liver-type actions to metabolize/detoxify drugs and xenobiotic substances that could harm the brain
- Kidney-type organ that purifies/buffers extracellular fluid composition (e.g., H and K ions) for neuronal networks
- Receiver of sex hormone signals (e.g., progesterone) to regulate choroid plexus functions, e.g., transthyretin synthesis/secretion for brain protein modulation
- Provider of polypeptides and proteins (e.g., cystatin C) for regulation of cerebral metabolism, and for mediating restoration of brain injury
- Sentinel-like function to detect neuroinflammation and injury-generated molecules in brain
- Intracranial pressure-regulating organ that responds to elevated ventricular pressure by hormonally (e.g., atrial natriuretic peptide) downregulating CSF formation rate
- Source of the CSF that provides the watery medium for volume distribution of molecules within the ventricles and to cortical subarachnoid regions (for entry into paravascular flow routes)
- Taste receptor sensing of CSF bitter/toxic compounds, hypothetically for homeostatically refining the chemical composition of brain extracellular fluid milieu

Figure 2: CSF formed by CP cells (green) flows down the ventricular system into subarachnoid space (SAS) surrounding cortex, cerebellum, brain stem and spinal cord. Ependymal cells (yellow) line the ventricle walls, providing a permeable interface between CSF and brain ECF. Astrocyte processes extend to ependyma and to capillary walls of BBB. CSF oscillates gently to-and-fro with cardio-respiratory rhythms while undergoing bulk flow (arrows) to distal reaches of SAS. CSF under pressure flows over the cortices to drain across arachnoid villi (AV) into venous blood (lavender), or to enter the brain’s paravascular system at the Virchow-Robin spaces (VRS). Bottom right: Exosome vesicles, containing folate, F (red), are released into CSF for transit to various parenchymal targets. MVB, multi-vesicular bodies GEEC, early endosomal compartments Adapted from ref. 44 (Grapp et al.)

and BBB, but the reader can find comprehensive treatises elsewhere7-10. Active Na distribution across membranes determines the fluid balancing between extracellular and intracellular compartments. It is timely to assess Na transporters that couple to \( H_2O \) movement. For the BCSFB and BBB, the Na pump (Na-K-ATPase) faces the CNS side of the barrier and establishes steep Na gradients across the low-[Na] barrier cells. Vigorous Na pumping is critical for cell volume, voltage gradients, and the net trans-barrier movement of organic solutes. Another major mechanism is the Na-K-Cl cotransporter, that promotes fluid movement and contributes to barrier cell [Cl] stability and CSF-ECF K homeostasis. A key to understanding cerebral and CP edema (e.g., in stroke, trauma and hyperthermia) is that fluid retention stems from compromised Na transport and reduced water removal. Conversely, Na-H\(_2\)O fluid movements at the barriers may increase in certain inflammatory states.
gradients that drive CSF formation. Mitochondrial failure, primary by gene mutation or secondary to amyloid-beta (Aβ) accumulation in CP as in Alzheimer’s disease, curtails CSF dynamics and disrupts solute homeostasis/gradients; cell energy failure from ATP diminution also enhances oxidative stress. Mitochondrial apoptosis, associated with disordered Sirtuin 1 gene function, increases in barrier injury and autoimmune disease. The mitochondrial division inhibitor, Mdivi-1, suppresses apoptosis and protects against barrier breakdown. Deficient mitochondrial (ATP-generating) function diminishes organic solute movements in CP, resulting in lower folate and higher homovanillic acid concentrations in CSF. Accordingly, correcting CSF-brain dyshomeostasis in neural disorders should include stabilizing mitochondrial function and energy state in barrier epithelium and endothelium.

Barrier alteration by inflammatory cytokines: triggering of neuroinflammation

In addition to the aforementioned bioenergetic deficiency problems, systemic inflammation pathophysiology also threatens the wellbeing of BBB-BCSFB. Barriers are inflamed by vascular disorders (stroke), traumatic brain injury, infections and byproducts...
of neurodegenerative disease, e.g., amyloid plaque. Disrupted transport interfaces provoke ECF biochemical instability and immune-brain imbalance in parenchyma of cortical, CSF and periventricular regions. Lipopolysaccharide (LPS), used in many experimental models of neurodegeneration, disrupts the BBB and BCSFB; consequently, upon penetrating the damaged barriers, LPS drives the NF-kB associated progressive inflammatory signaling in the parenchyma. This is a prominent feature of neurodegeneration.

Perturbed immune-brain interaction underlies many neurological/psychiatric disorders: status epilepticus, stroke/ischemia, and the neurodegenerative Parkinson’s and Alzheimer’s diseases. Neurons malfunction when overrun with ECF perturbations. To heal both the barrier elements and parenchymal cells, the BBB and BCSFB in injury states compensatorily secrete peptides, growth factors and neurotrophins. These beneficial factors stabilize extracellular-intracellular fluid balance for neural health. Various modulating factors, presented in Table 2, are addressed in topics below.

Several brain disorders discussed in later sections are caused/exacerbated by disrupted barriers that encompass neuronal networks. Frequently, barrier opening precedes clinical symptoms. Barrier breaching enables central invasion by foreign substances, microbial agents and immune elements. Consequently, ECF composition is tainted by the centrally-invading plasma proteins and immune elements normally screened by barriers. Sentinel-like detection and resolution of neuroinflammation depend on an intact CP and neurovascular unit.

Seizures stem from BBB opening in cerebral tumors, trauma and stroke. BCSFB permeability increases after stroke or mechanical injury, predisposing to seizures. Stabilizing the compromised CNS barriers, to suppress neuroinflammatory progression and toxic immune reactions, significantly restores neuronal excitability and thwarts seizures. An electroconvulsive model reveals upregulated growth factors, including erythropoietin, and trophic proteins in CP responding to seizures. Hormonal erythropoietin exogenously administered (or induced in CP) holds promise for augmenting BCSFB regulators to foster CP-CSF-brain homeostasis. Such positive actions by erythropoietin and other trophins reduce CSF oxidants, attenuate neuroinflammation, and lower depression as well as seizure activity.

Table 2. Choroid plexus hormones, neurotrophins, and growth factors: transported/secreted into CSF to support BCSFB and multiple brain regions

<table>
<thead>
<tr>
<th>Hormones transported/distributed by CP from blood to CSF</th>
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<tbody>
<tr>
<td>Leptin, an appetite and metabolism regulator</td>
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<tr>
<td>Prolactin, a reproductive hormone and lactotroph</td>
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<tr>
<td>Adipsin and adiponecin, fat hormones that regulate neuroinflammation</td>
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<tr>
<td>Orexin A or hypocretin-1, an arousal and appetite hormone</td>
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<tr>
<th>Neurotrophins/neurotrophic factors synthesized/secreted by CP into CSF</th>
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<tr>
<td>Brain derived neuronal factor (BDNF)</td>
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<tr>
<td>Gial derived neuronal factor (GDNF)</td>
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<tr>
<td>Neurotrophin-3 (NT-3)</td>
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<tr>
<td>Neurotrophin-4 (NT-4)</td>
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<td>Ciliary neurotrophic factor (CNTF)</td>
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<tr>
<th>Growth factors transported/distributed by CP from blood to CSF</th>
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<tbody>
<tr>
<td>Insulin-like growth factor-1 (IGF-1)</td>
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<tr>
<td>Hormones synthesized/secreted by CP into CSF</td>
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<tr>
<td>Arginine vasopressin (AVP) fluid regulator</td>
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<tr>
<td>Augurin, an ecrg4 product that regulates mitosis</td>
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<tr>
<td>Hecipidin, an iron regulator</td>
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<th>Growth factors synthesized/secreted by CP</th>
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<tr>
<td>Nerve growth factor (NGF)</td>
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<tr>
<td>Basic fibroblast growth factor-1 (FGF-1)</td>
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<tr>
<td>Acidic fibroblast growth factor-1 (FGF-2)</td>
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<tr>
<td>Transforming growth factor beta (TGFβ1; β2; β3) and TGFβ latent binding proteins (LTBP-1 and -3)</td>
</tr>
<tr>
<td>Hepatocyte growth factor (HGF)</td>
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<tr>
<td>Insulin-like growth factor-2 (IGF-2) and binding protein</td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF) and VEGFC ligand</td>
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<tr>
<td>Granulocyte colony-stimulating factor (G-CSF), an hematopoietic factor</td>
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<tr>
<td>Amphiregulin, a member of epidermal growth factor family</td>
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Figure 4: Tight junctions (J) weld adjacent choroid cells at their CSF-touching loci. Narrow paracellular pathways, just under the tight junctions, finely separate adjacent cell lateral membranes. Extensive microvilli (Mv) on apical membrane confer extensive surface area for transport processes. Abundant mitochondria (M), endoplasmic reticulum (ER) and Golgi apparatus (G) support extensive protein synthesis and solute transport, and energize high rates of metabolism and CSF formation. The basal labyrinth (BL) maximizes transport area by intertwining apposing membranes of adjacent choroid epithelial cells. C, centrioles Nu, nucleus Adult rat lateral ventricle CP was fixed with OsO₄ for electron microscopy. Scale bar (bottom, right) = 2 µm
Cells of the BCSFB and BBB secrete neurotrophins, immunoregulators (interferons), and anti-inflammatory cytokines for CSF adaptation to circulating vascular stressors. Accordingly, the brain endothelium and choroidal epithelium ‘fight back’ against the systemic inflammation that immunologically irritates transport interfaces. Severe systemic inflammation threatens BCSFB and BBB integrity as barriers become greatly permeabilized with dire consequences for brain. Peripheral inflammation shifts the ECF to a pro-inflammatory state. A popular method to create a pro-inflammatory environment in the CNS is by injecting the bacterial LPS peripherally so that LPS/TLR4 receptors at the BBB-BCSFB are stimulated to produce ‘inflammatory responses’ within the barrier elements. This local inflammatory response, i.e., cytokine production in barrier cells, is then transmitted outward into brain ECF and CSF.

BBB injury from cytokine upregulation accompanies most brain diseases/infections. LPS ‘challenge’ has been useful for experimentally ‘dissecting’ components of inflammatory damage to brain microvessels. Plasma cytokines/microbial products, via toll-like receptor stimulation, transduce barrier targets to express immunoregulatory molecules for secretion. There are multiple reports of LPS consistently increasing BBB permeability and vesicular activity, and harming cerebral endothelium; adverse effects on glycolcyti, glia limitans and astrocytes also eventuate from LPS administration. In disease (vs. health) the BBB is more sensitive to systemic inflammation.

LPS disruption of BBB permeability is variable, depending on LPS dose, regional effects and neurochemical factors. LPS-induced global BBB breakdown is less severe than that by focal cold injury. Banks et al. demonstrated that LPS enhances BBB leakiness to 14C-sucrose and albumin in several regions, but not in hypothalamus even at a very high dose. Pathophysiologic state matters for LPS actions. LPS intensifies BBB opening in animals with acute liver failure. Interestingly, synuclein expression increases the BBB ‘leakiness response’ to LPS stimulation. Clearly, LPS compromises BBB integrity, an effect amplified by various diseases. The anti-oxidant melatonin experimentally counters LPS-induced damage to BBB and CP.

The BCSFB also responds sensitively to circulating LPS. Over 400 CP genes are differentially expressed following LPS injection of mice; upregulation clusters into immunoregulating and extracellular matrix-remodeling genes, while downregulation results in less control of barrier maintenance. This transcriptome alteration centers around the so-called acute-phase response to peripheral inflammation. CP is ideally situated, with the help of CSF which carries the secreted proteins, to pass immunosignaling molecules into the ventricle-brain axis.

In the relatively weak neuroimmune response of ‘sickness behavior’, cells at the CNS-immune interface (CP epithelial/stromal cells, leptomeninges, and hippocampal neurovascular cells) are ‘initial responders’ to LPS injected into mice. Thus, in sickness behavior, cytokine production in barrier cells (4 hr post-LPS endotoxemia) precedes the spread of barrier-generated immune signals by ECF. Later, responding to barrier-produced and -distributed cytokines, hippocampal astrocytes secrete cytokines that expand the CNS immune reaction to the original source of peripheral endotoxin.

A more virulent disorder than sickness behavior is the sepsis-associated encephalopathy (SAE). SAE varies in pathologic severity but does not necessarily involve CNS bacterial invasion; this disorder carries high morbidity/mortality and is characterized by neuroinflammation, circulatory dysfunctions, CNS barrier breakdown and cognitive impairments. Long-term BBB dysfunction (MRI evaluation) augments cerebral metabolic distortions and modifies neurobehavior in the LPS-induced SAE model. BCSFB is also hit hard by experimental sepsis, engendered by either LPS i.p. or cecal ligation/puncture; upregulated matrix metalloproteinase 8 (MMP8) is a key factor in disrupting CP extracellular matrix and epithelial structure. The greatly compromised BCSFB significantly contributes to the subsequent development of damaging neuroinflammation. Due to potential lethality, the systemic inflammatory response that destroys CNS barriers is aseptic research area urgently in need of pharmacotherapeutic remedies. Nanobody agents that counter sepsis-induced MMP8 activity particularly for BCSFB application, may help minimize sepsis/inflammatory damage to the CP-CSF-brain nexus.

LPS continues to be useful in stimulating/characterizing CP immune phenomena. That is, bacterial molecules stimulate CP epithelial receptors, generating immunoproducts and vesicles for transfer into CNS. Low-grade systemic infection or LPS/TNFα administration prompts CP to form vesicles (exosomes) containing small RNA molecules. CP discharges these vesicles into CSF, presumably for regulating astroglial/microglial targets. CSF bulk flow promotes vesicle distribution throughout brain and spinal cord. Vesicle dynamics also raises therapeutic possibilities for improving CP-to-neuron communication (signaling). Barrier-generated vesicles, their composition and distribution kinetics, deserve further investigation. Exosome inhibition of CP vesicle release has potential application for controlling neuroinflammation. Pharmacologic use of CSF-borne vesicles as non-immunogenic drug (and RNA) carriers holds promise for next generation cell-biological repair of the dysregulated cerebral metabolism and neuroimmunity in neurodegeneration.
The sections below expand upon previously integrated CP-BBB data\textsuperscript{47}, by updating disordered homeostatic mechanisms in the CSF-brain nexus. For wide-ranging pathophysiologies, the relative roles of each barrier are examined. We emphasize that in certain disorders the CP is the main barrier affected. In others, BBB also fails. A recurring theme is that brain recovery requires reinstated dual-barrier stability for strong ECF homeostasis.

**Separate vs. Overlapping Barrier Functions**

Stabilizing functions work jointly, but sometimes separately by BBB or BCSFB. Cerebral capillaries and choroid plexuses remove from extracellular fluid excesses of various solutes. These include clearing superfluous K, inorganic anions (e.g., iodide and thiocyanate) and organic anions (e.g., 5-hydroxyindole acetic acid and homovanillic acid catabolites of biogenic amines). Certain peptides are reabsorbed into blood by BBB or BCSFB, but there are regional differences in transporter expression. Thus, the Pept2 transporter in apical membrane of CP (but not BBB) removes small peptides from CSF, e.g., the dipeptide alanine-alanine\textsuperscript{48}. Transport analysis of the disaccharide glycylsarcosine in isolated mouse CP in which the \textit{pept2} gene was completely ablated reveals Pept2 as the main member of the peptide transporter family that reabsorbs dipeptides at the BCSFB\textsuperscript{49}.

Other efflux systems, such as the multidrug resistance protein (MRP), organic anion transporter (OAT), and organic anion transporting polypeptide (OATP), are present in both BBB and BCSFB\textsuperscript{48}. These clearance transporters remove organic anions from brain ECF and CSF. Cephadroxil, an antibiotic, is avidly removed from central ECF by MRP, OAT and OATP. Such removal from brain-CSF by these transporters may lower the agent concentration to sub-therapeutic levels in the CSF. Preventing undesired reabsorption, i.e., efflux from CNS, comes about with competitively-inhibiting drugs such as probenecid. Neurodegeneration and neuroinflammation disrupt CNS barrier transporters. Such barrier disruption adversely affects ECF homeostasis, disease progression and neural pharmacotherapy\textsuperscript{50}.

On the CSF \textit{influx} side, several endogenous compounds penetrate substantially by the BCSFB. CP acts as a ‘relay station’ in transporting hormones from blood to hypothalamus. These include prolactin and insulin-like growth factor 1 secretion, from plasma-to-CP-to-CSF. Hormones then flow by volume transmission (Fig. 5) to hypothalamic target cells\textsuperscript{51}. Vitamin C is transported into CNS via the ascorbate SVCT2 transporter in CP epithelium\textsuperscript{52}, but not BBB endothelium; accordingly, SVCT2 knockout at the BCSFB severely depletes brain ascorbate\textsuperscript{53}. Major transport of folate across BCSFB is essential for proper neural tube development; conditions associated with marked CSF folate dwindling lead to spina bifida/hydrocephalus. Transthyretin, secreted by CP epithelium but not cerebral endothelium, facilitates thyroid hormone transport into CSF-CNS and protects neurons by stabilizing amyloid conformation\textsuperscript{54}. Aquaporin 1 (AQP1) at the BCSFB facilitates water penetration into CSF\textsuperscript{55}. AQP1 is not normally in the BBB, which instead expresses AQP4 in astrocyte feet to enhance water fluxes between plasma and brain.

Organic metabolites, e.g., creatinine/urea, and cytokines interact with both barriers\textsuperscript{56,57}. Certain immune phenomena, e.g., leukocyte migration, may associate more prominently with BCSFB than BBB. Under particular conditions, the CP gate allows leukocyte penetration to downstream periventricular regions\textsuperscript{58}. This occurs in multiple sclerosis relapse. Viruses, bacteria and fungi exploit CP as a CSF ‘port of entry’ into brain\textsuperscript{59,60}. HIV uses CP interstitial milieu (Fig. 3) to promote evolution of drug-resistant viral strains\textsuperscript{61}, with untoward consequences for CSF that contacts multiple CNS regions.

Proteins leak from blood into brain and CSF. This ‘proteinaceous’ infiltration increases in aging\textsuperscript{62} and
neurodegeneration. Proteins diffuse across BCSFB more extensively than BBB, due to more permeable tight junctions in CP more than cortical capillaries. Accordingly, in neural diseases the CSF-to-serum ratios of proteins such as albumin (mw ~68,000) primarily reflect BCSFB breaches. This concept needs to replace the established inaccurate notion that the CSF-to-serum ratio of proteins mainly assesses brain microvessel permeability.

Our review highlights the spawning of new concepts on regulated interfaces separating blood, CSF and brain. Multi-faceted regulators at CP epithelium and cerebral microvessels safeguard neuronal milieu. Many polypeptides and proteins, arising from BCSFB transport into CSF, modulate brain viability. Synaptic environments suffer upon failure of homeostatic transport mechanisms at the barriers. Hence, following severely-perturbed CSF-brain homeostasis, neurotransmission falters. Cognitive and behavioral losses ensue. Extensive barrier trauma leads to morbidity crises and even death. Therefore, novel strategies are sought to safeguard barrier systems or quickly restore their function.

A continuing concern is the BBB’s role in CNS impairment. Much focus is on neurovascular unit failure in trauma, ischemia and neurodegeneration. We now integrate data primarily for BCSFB damage, but also for concurrent breakdowns of CP and BBB. Our treatment covers new stratagems with CP-CSF pharmacology to restore compromised barriers.

Historical vs. Current Paradigms of Barrier Investigations

Past research overstated BBB importance and obscured the BCSFB role. A PubMed search in May 2018 revealed ~44,000 articles for BBB and ~10,000 for BCSFB/CP. Attention directed to BCSFB malfunctions prompts novel possibilities for translational research. In reality, both systems normally operate together. Their actions therefore should be integrated in models of neural homeostasis and disease.

Brain is broadly immersed in CSF, and CP forms most of the CSF. It follows that the villous CP plays a chief role in central homeostasis (Fig. 2). CP floats in ventricular CSF, spreading out like a fishnet. This maximizes surface area for microvilli exchange (Fig. 3). To-and-fro movement of CSF, responding to cardiac and respiratory pulsations, physically displaces CP villi. This allows thorough mixing of CSF for monitoring and adjustments. Currently undergoing revision is the historically inaccurate perception of CP as a ‘minor player’ due to small surface area. BCSFB is currently regarded as a highly dynamic transport interface with activity intensity on par with BBB. This notion is bolstered by findings that CP-generated net turnover of CSF greatly exceeds BBB fluid generation.

CP as a multi-functional organ has been stressed over recent decades. Its wide array of actions/reactions benefits the brain and spinal cord (Table 1). An interesting liver-like analogy is CP epithelial glutathione detoxification to protect neurons. A kidney-like function is the newly-discovered Cl-/H+ exchanger in CP luminal membrane. Cl-/H+ exchange is being explored for CSF pH and acid-base regulation. Multiple taste receptors are expressed in CP epithelial cells (Tas1r1, Tas1r2, Tas1r3, Tas2r109 and Tas2r144); transduction of their signals is consistent with a putative role of tasting bitter, noxious compounds in CSF. The role of taste signaling at the BCSFB, possibly in regard to refining the neural milieu, awaits elucidation.

Newly-described choroidal functions stimulate thinking for future CP-CSF modeling. Recent findings on CP internal housekeeping includes syncytial actions that tie together, by gap junction communication, periodicity signals affecting extra-choroidal regions. Physiologic rhythms mediated by the hormonally-regulated CP circadian clock, via CSF delivery of cytokine signals, the ‘master coordinator’ circadian clock in the hypothalamic suprachiasmatic nucleus.

The prospect of using specific bile products, e.g., tauroursodeoxycholic acid, in neurological disease management is a captivating innovation on the horizon. Stimulation of brain cell receptors by deoxycholic acid derivatives restores mitochondrial function and ATP energy levels. This protects against experimental neurodegeneration advancement in certain Parkinson models. Nrf2, short for nuclear factor-erythroid 2-p45 derived factor, is a master regulator protein that counters free radicals by stimulating several anti-oxidative enzyme systems. Upregulated transcription factor Nrf2, by tauroursodeoxycholic acid and isothiocyanates, protects dopaminergic neurons and BCSFB against excessive oxidation (as in neurodegenerative stress). This Nrf2 protective effect is mediated by a rise in the anti-oxidant enzymes hemeoxygenase 1, glutathione peroxidase and quinine oxidoreductase. Consequently, induced Nrf2 expression protects CP and dopaminergic neurons by preventing progressive cytotoxic damage. The isothiocyanates, sulforathane and allyl-isothiocyanate, from cruciferous vegetables also stimulate Nrf2 expression, thereby protecting CP from oxidative disruption. Nrf2 augmentation by tauroursodeoxycholate and isothiocyanates is a putative pharmacological target to strengthen CP homeostatic reserve and nigral neuron viability.

Another recent, intriguing BCSFB discovery, involving peripheral organs, is bacterial infection interactions between CP and the gastro-intestinal tract. Gut inflammation leads to neurodegeneration putatively linked to BCSFB breaching. Such CP inflammation relates...
to gastric mucosal infection spread into the CNS. Thus, CP-CSF functional interactions include peripheral phenomena as well as intra-brain homeostatic adjustments.

Now we elaborate structural/functional alterations of barriers that distort fluid parameters in neuronal and glial networks. First, we treat recently-reported phenomena transpiring mainly at CP. Thereafter we introduce new concepts on concurrent actions, beneficial and adverse, at the BCSFB and BBB. Evaluating the respective barrier roles in a range of pathologies reveals precise pharmacologic targets to reconstitute injured brain. Evidence is organized below advocating for an approach to target the BCSFB with medicinals that heal the epithelium damaged by microbes and other agents. A worthy therapeutic goal is to alleviate major CNS disorders triggered primarily by microbes and other agents. A worthy therapeutic goal is to alleviate major CNS disorders triggered primarily by CP malfunction, as a substantial mechanism (apart from to alleviate major CNS disorders triggered primarily by CP malfunction, as a substantial mechanism (apart from CSF functional interactions include peripheral phenomena as well as intra-brain homeostatic adjustments.

Barrier-Interface Actions Localized Mainly to Choroid Plexus

Strong evidence implicates CP-CSF-brain solute transport and distribution as critical for CNS ontogeny. Malfunctions of transport systems and CSF volume distribution (as in congenital hydrocephalus) predispose to developmental neural diseases/disorders. Investigators increasingly analyze both barriers simultaneously to devise integrative transport models. Such modeling will likely find application in neurotherapeutics. Below we localize the onset of neural disorders to BCSFB disablements. CP functional diversity, when disrupted, gives rise to a wide spectrum of CSF-brain pathophysiology. Translational opportunities deserve exploration to counter impairments (discussed below) that selectively afflict CP interstitial-epithelial zones.

Systemic lupus erythematosus

Neuropsychiatric systemic lupus erythematosus (NPSLE) is an autoimmune disease caused/exacerbated by leukocyte and immunoglobulin penetration into CSF, then to periventricular regions that become inflamed. Formerly, a leaky BBB was implicated in NPSLE onset; however, a paradigm shift in transport modeling may be emerging. Gelb and colleagues tested an SLE mouse model (MRL/lpr) for penetration of permeability probes: immunoglobulin G, dextran (mw 10,000), and horseradish peroxidase (HRP). These large-sized probes and lymphocytes did not penetrate BBB but readily crossed CP via transcytosis (not tight junctions). Thus Gelb et al. challenge the disrupted BBB model of NPSLE, concluding that antibodies/lymphocytes permeate a perturbed BCSFB en route to inflaming the subependymal regions. To explain NPSLE, they propose shifting the experimental focus to CP-CSF.

N-methyl D-Aspartate-associated autoimmune encephalitis

Autoantibodies to N-methyl D-Aspartate (NMDA) receptors wreak havoc on neurotransmission in autoimmune encephalitis. An imbalanced inflammatome promotes neuroinflammation-mediated cognitive decline. Autoantibodies against NMDA receptor are in CSF. Still, studies to attribute the autoantibody penetration centrally to the BBB have not been fruitful. Hammer et al., for example, found that intravenous NMDA receptor antibody did not penetrate a healthy BBB.

BCSFB is more permeable than BBB, especially in neurodegeneration. Thus, it makes sense to determine if CP in MCI/Alzheimer's disease allows enhanced CSF penetration of NMDA receptor antibodies. Recent verification of augmented BCSFB permeability to NMDA (elevated CSF/serum albumin) also found increased NMDA receptor autoantibody in CSF. The Busse et al. findings reveal that certain patients transmit more NMDA receptor antibodies into CSF as neurodegeneration worsens. This subset points to a dual problem: a) the compromised general function of BCSFB in Alzheimer's disease; then b) allows more serum autoantibodies to reach CSF-brain. Therapeutic targeting and stabilization of a) above may attenuate pathophysiology associated with b).

Helicobacter bacterial disruption of choroid plexus

Gut microbiota profile shifting is linked to disorders such as Parkinson's disease. This linkage includes CP inflammation in pathophysiologic pathways. Bacterial infection by Helicobacter suis permeabilizes the gastrointestinal tract; in mice, this leads to systemic infection that spreads to CNS causing microglial activation. Helicobacter s. invades human CP epithelium, from the basolateral side. Information is needed for vagal signal contribution, and time points as well as permeability measures for this CNS disturbance.

The Helicobacter s. inflammation and dysfunction observed in BCSFB are not found in endothelial BBB. Helicobacter disablement of BCSFB specifically identifies CP as a 'causality' in stomach infection disorders giving rise to CSF-brain inflammation that hurts cognition. Thwarting Helicobacter's adverse effects on CP may help stave off cognitive and neurodegenerative problems stemming from gastrointestinal microbiotic disorders.

Choroidal toll-like receptor activity and neuroinflammation

Central conditions that inappropriately stimulate toll-like receptors devastate neural functions. Infection-induced inflammation in early life gives rise to maldevelopmental issues such as cerebral palsy. Adjusted
leukocyte/cytokine migration across BCSFB, controlled by toll-like receptor activity, is a significant aspect of the innate immune response (pathogen pattern recognition). Toll-like receptors (TLR) in CP consist of 9 identified transcripts for receptor subtypes. TLR2 activity promotes neuroinflammation and neuronal damage. Clinicians need a better understanding of TLR2 impact on BCSFB permeability and leukocyte movement into CSF.

TLR2 stimulation by the synthetic agonist Pam3CSK4 (PC3) increases permeation of monocytes and neutrophils into CSF. PC3 also augments BCSFB permeability to sucrose. CP inflammation provoked by LPS is substantially less than that by PC3. Low levels of endotoxic LPS in plasma induce cytokine signals in CP that migrate to CSF and brain (Fig. 6). This signaling apprises the CNS about peripheral inflammation. LPS also upregulates TLR2 in CP, and induces pro-inflammatory cytokines. Accordingly, even without pathogen access to CNS, the protective innate immune response is generated in microglia from signals received via CP-CSF.

Specific activity of TLR2, but not TLR4, promotes neutrophil and monocyte infiltration into the CSF and downstream arachnoid membrane. This is mediated largely by the BCSFB. Such leukocyte trafficking prompts exploration with synthetic lipopeptides on how inflammatory reactions hurt the developing brain. TLR2 holds special interest in analyzing lipopeptide-driven neurodegeneration. Antagonizing toll receptor activity at BCSFB may limit harm by TLR2-associated neuroinflammation on vulnerable regions.

**Choroid plexus-CSF gateway for trypanosomes**

Trypanosome inducers of African sleeping sickness initially and rapidly enter CSF, but not brain, after intraperitoneal injection. *Trypanosoma brucei* readily passes through the BCSFB, en route to eventually infecting brain following passage via ventricular CSF flow into SAS (Figs. 2 and 6). This microbe accesses the Virchow-Robin spaces, that are entry loci for subarachnoid CSF flow into cerebral paravascular spaces. (Trypanosomes, activators of lymphocytes, also extensively accumulate in the dura, likely gaining access to dura mater from the blood side.) Trypanosome counts in blood-free CSF are cyclical and proportional to trypanosome infection density of the blood.

There is meager evidence that trypanosomes pass directly and extensively across BBB. This needs resolution however by newer, sensitive methodologies. To date the best evidence is that trypanosomes penetrate the CNS predominantly by initial CSF access, following which there is convective distribution into brain to set up the second stage of African sleeping sickness. A potential pharmacologic control point for attenuating CNS damage is to block passage of trypanosomes through BCSFB.

**Figure 6:** Outward spreading of signaling cytokines into CSF-brain, following serum immune molecule stimulation of CP cytokine receptors TLR2 and CD4. When stimulated by serum immune molecules, e.g., LPS (white arrows), toll like receptors in CP (and other CSF-bordering cells) respond by ‘transducing’ the peripheral signal into synthesis of epithelial cytokines. Receptors for TLR2, TL4 and CD4 expressed in CP and circumventricular organs receive cytokine signals from immune elements in blood perfusing the plexus. Some of the newly-manufactured cytokines in the choroid cell, as part of the innate immune response, are released (gray arrows) into ventricular CSF. Volume transmission conveys cytokine signals in CSF to brain targets (curved black arrows) in various regions. Thus, CNS (e.g., microglia) is apprised of peripheral immune status, without the cytokine, leukocyte or microbe in serum penetrating the BCSFB. Adapted from Johanson et al., Pharm. Res. 2005; 22:1011-37.

**Cholesterol and choroidal transport of amyloid**

Knowledge of Alzheimer pathophysiology is more complex than the original concept of neuronal Aβ production vs. BBB clearance. It is unlikely that any single factor is explanatory. Other complicating factors for neurodegeneration are under consideration both at the systemic (glymphatic drainage) and molecular level (cholesterol). The glymphatic system, involving astrocyte transport, is a “front end” for waste clearance; it is connected downstream to a valid network in the cervical lymphatics. Impaired drainage of Aβ in glymphatic fluid into cervical lymph is likely a contributing factor in Alzheimer pathophysiology, but awaits corroboration.

Aβ distribution within CNS relates to cholesterol homeostasis and transport at the barriers. Imbalanced cholesterol metabolism/transport in CP-CSF may harm brain. APOE regulates lipid distribution between plasma and body compartments, e.g., CSF and brain. Free cholesterol exchange across BBB and BCSFB favorably affects net Aβ extrusion from CNS. Individuals hetero- or homozygous for the APOE4 allele have greater vulnerability for disrupted barriers.

In choroidal epithelium, cholesterol release across
the apical membrane into CSF is less efficient by APOE4 modulation than APOE3\textsuperscript{100}. Less APOE4-dependent extrusion leads to cholesterol accumulation in choroidal epithelium, possibly harming BCSFB functions. Aβ concentration stability in CNS depends on cholesterol homeostasis. It seems worthwhile to identify Aβ and cholesterol-stabilizing drugs, such as bevarotene (a stimulator of APOE4-mediated BBB cholesterol efflux)\textsuperscript{39}, for moderating Alzheimer's disease. Improving CP-CSF BBB cholesterol transport may help to limit Aβ-linked neurodegeneration\textsuperscript{100}.

**Concurrent Actions Associated with BBB and BCSFB**

How best to assess the permeability of substances distributing across cerebral capillaries into interstitium vs. those through CP into CSF? Uptake of Evans blue-albumin complex by brain shortly after intravenous injection reliably evaluates barrier leakiness. In clinical analysis of dementia, enhanced CSF/SER for albumin is equated to augmented 'BBB permeability'\textsuperscript{101}. Almost certainly, some albumin in brain interstitium (from BBB leakage) diffuses into CSF. Still, steady-state CSF/serum albumin values primarily reflect BCSFB permeability\textsuperscript{64}.

Although some investigators lump brain capillaries and CP together generically under the 'BBB' category, this is inappropriate because of the fundamentally different properties of these endothelial vs. epithelial barriers. Accordingly, BBB refers specifically to the cerebral microvessel wall, whereas BCSFB typically designates the CP epithelial membrane.

Discussed below are studies demonstrating altered BCSFB and BBB, occurring to different degrees in the various regional analyses. Time course and severity of barrier modifications impact disease progression as well as remediation.

**Helminthic meningitis**

The BCSFB undergoes severe disruption in lungworm-induced meningitis. This is contracted by ingesting raw snails with larvae of *Angiostrongylus cantonensis*. Centrally, a cardinal feature of this disorder is inflamed, impaired CP. CSF has an elevated eosinophil count. Eosinophilic inflammation disrupts BCSFB\textsuperscript{102}, increasing CSF protein, albumin, plasminogen activator, matrix metalloproteinases (MMP-9 & MMP-12), TNFα and claudin-5. Inflammatory molecules and eosinophils flow via ventricular CSF outward to the distal meninges. Serious infection of the arachnoid membrane usually results.

Choroid plexus disruption is central to this helminthic disorder. Eosinophils and cytokines, e.g., TNFα, activate molecular pathways at the BCSFB. This leads to NF-kB stimulated matrix metalloproteinase MMP-9. Claudin-5 tight junctions disassemble; enhanced paracellular permeability ensues. Experimental inhibition of NF-kB/MMP-9 cascading attenuates BCSFB breakdown and reduces CSF protein, leukocyte number, cytokines, and other CP-derived injury molecules\textsuperscript{103}. MMP-knockout animals have less perturbed CSF, reduced brain immune cell aggregates, and less virulent meningitis from *Angiostrongylus* infection\textsuperscript{104}. Augmented hepatic growth factor in CP-CSF\textsuperscript{105} likely promotes BCSFB repair in the subsiding infection.

That the BBB is also injured in this meningitis-encephalitis disorder is revealed by augmented MRI signals\textsuperscript{106} and Evans blue staining of animal-model brains. Additional investigations, however, should characterize effects of *Angiostrongylus c.* on the cerebrovasculature altered by eosinophilia reactive to larvae. Resveratrol, corticosteroids and anti-helminthic agents reverse CP damage and CSF levels of elevated cytokines\textsuperscript{107}. Most CNS knowledge on this meningitis disorder comes from CSF parameters, largely reflecting BCSFB pathophysiology. Pharmacology efforts to control lungworm-associated neuropathology should include drug-manipulated CP.

**Ischemia/stroke damage to the barriers**

Reduced CP perfusion exacts a rapid, heavy toll on the BCSFB. Hippocampal CA regions protected by BBB also incur great damage, by a delayed response (at 48 hr). When highly-metabolizing CP is deprived of O\textsubscript{2}, the BCSFB epithelial syncytium disintegrates. In addition to traumatizing the hippocampal CA regions\textsuperscript{52}, transient forebrain ischemia devastates rat CP epithelial lining\textsuperscript{20}. Obliterated microvilli, swollen barrier cells, and formed cytoplasmic vacuoles (from 10 min of interrupted blood flow and reperfusion) manifest the CP disintegration\textsuperscript{20}. Remarkably, the ischemia-injured epithelium recovers by 24 hr. This remarkable recovery is aided by trophic/healing effects of hormones and growth factors secreted by CP\textsuperscript{107} (Table 2).

A breached BCSFB, reflected by a 3-fold increase in \textsuperscript{3}H-inulin permeability, was noted by Ennis et al. at hour 6 following vessel-occlusion ischemia. They posited that neuronal death in hippocampus at 48 hr was worsened by BCSFB leakiness shortly after stroke/ischemia induction\textsuperscript{108}. This notion is supported by the observation that, during ongoing transient forebrain ischemia, apoptosis in rat CP precedes neuron death in CA\textsubscript{1109}. This temporal sequence fits the stroke model of delayed hippocampal injury relating to the earlier-occurring harm of the nearby BCSFB. Still, factors secreted by CP, such as cystatin C protease inhibitor, likely counter certain hippocampal damage\textsuperscript{110}.

How is hippocampus impacted by altered CP transport? Stroke-induced breaching of the BCSFB opens up a 'pathophysiologic portal' through which proteins, cytokines and unneeded plasma molecules freely pass into
the ventricles. Water osmotically follows molecules leaking into CSF. Ventriculomegaly results; and brain regions proximate to CSF are destabilized. An inflammatory stimulus, co-existing with ischemia/hypoxia, may exacerbate stroke damage by down-regulating the CP Nrf2 anti-oxidant system.

Stroke harm to BCSFB prompts plasma-to-CP-to-CSF homeostatic trafficking of monocyte-induced macrophages. When anti-inflammatory M2 macrophages are injected into CSF, the adverse impact of stroke on mouse CNS decreases. M2 trafficking across CP into CSF also heals spinal cord injuries distant from BCSFB. Evidence supports a beneficial CSF flow of M2 cells from ventricular CP secretion to downstream regions of SAS. Regimens to increase M2 cells in CSF appear promising to calm inflammation, even in spinal injuries.

**Hyperthermia and disrupted barrier integrity**

Elevated temperature increases BCSFB-BBB permeability, likely by rupturing tight junctions between barrier cells. Whole body hyperthermia (38°C) for 2 hr in rats opens the BBB to trypan blue, causing edema in hippocampus, hypothalamus, caudate nucleus and thalamus. Simultaneous untoward effects on CP-CSF include choroid cell degeneration, lateral ventriculomegaly and periventricular neuropil destabilization. BBB and BCSFB damage from acute hyperthermia spreads widely in CSF-brain (Fig. 7).

Post-hyperthermia repair includes supportive choroidal growth factors and neurotrophins. CSF flow distributes restorative peptides throughout the ventricles and adjacent brain. Recovery from hyperthermia-induced neuron damage and edema results from ventricular infusion of brain derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1) and glial derived neurotrophic factor (GDNF). Hyperthermia effects on CP-CSF-brain mimic cortical trauma. Cerebroyisin (a mixture of peptides), upon CSF administration, heals the BCSFB.

Hyperthermia also increases BBB leakiness in other models. An alternating magnetic-field applied to MDCK cells in a transwell enhances paracellular uptake of nanoparticles. Pulsed-wave low-dose ultrasound hyperthermia applied to mice also increases nanodrug uptake by brain; this technique expedites permeation of anti-cancer drugs across BBB. Elevated temperature likely downregulates claudin proteins. Reduced claudin-1 expression occurs in the in vitro microvascular endothelial cell preparation at 39°C (vs. 37°C sham control). Conversely, hypothermia in rats (4 hr; 33°C) upregulates tight junction claudin 5; this reduces BBB damage from endothelium-harming insults. Clearly, pharmacologically-preserved tight junction proteins could well be a clinical strategy to minimize barrier breakdown in hyperthermia and other disorders. Tight junction targeting is also relevant for CP.

**Central leptin hormonal resistance and obesity**

Leptin upregulates in response to abdominal fat overload. Secreted by adipocytes, leptin targets the hypothalamus for neuroendocrine signal integration of energy metabolism and thermogenesis. Leptin's role in food intake and weight control commands great interest. The leptin receptor was first cloned for CP.

Leptin accesses the CNS by high-affinity transport at the BBB and BCSFB. Endothelial megalin expression facilitates leptin (and insulin) transport into brain to control obesity. A saturable carrier moves leptin across BCSFB into the ventricles; megalin (LRP-2) binding mediates transport. Leptin flows via CSF streaming to the hypothalamic arcuate nucleus. A second route for leptin to the arcuate is by saturable BBB transport at the capillary interface. Do both routes cover similar cell populations in hypothalamus? The need for two delivery pathways for plasma and CSF leptin to access the arcuate nucleus awaits elucidation.

Leptin’s modulation of arcuate neurons helps body weight control by feedback for upregulated adipocyte lipolysis. Resistance to leptin as a weight-regulating hormone is in two forms: i) impaired leptin transport...
across BBB and/or BCSFB into hypothalamus, and ii) reduced sensitivity of leptin receptors and diminished arcuate signaling. Leptin resistance in pregnancy, manifesting as hyperphagia (increased food intake), results from decreased leptin transport across barriers (transwell analysis) and from altered hypothalamic signaling. The central appetite-enhancing activity in lactation functionally relates to prolactin modulation of arcuate neurons.

On the other hand, food ingestion is spontaneously less in Lou/C rats. Hypophagia in Lou/C is not due to enhanced expression of CP leptin receptors. Triglyceride changes suggest augmented leptin transport across BBB. Lou/C rats also display improved hypothalamic signaling (increased pSTAT3/STAT3) and less leptin down-regulating cytokine signaling 3 (SOCS3).

Leptin actions intertwine with carbohydrate and fat metabolism. Hyperglycemia likely upregulates barrier leptin transporters, while hypoglycemia downregulates. Chronic early-life stress (mice) increases CP leptin mRNA. Putatively this is central adaptation to early-life stress, with ties to obesity vulnerability persisting into adulthood. Rats with leptin receptor (db/db gene) knockout by CRISPR/Cas9 display an obesity phenotype and carbohydrate distortions. Neutralizing antibodies, e.g., 9F8 against ObR leptin receptor, reveal that ObR may not be the primary leptin transporter in BBB (endothelial monolayer testing). Ablation by CRISPR/KnockOut and antibody neutralization should elucidate leptin physiology/pharmacology.

CNS barriers to leptin uptake, i.e., a form of leptin resistance, are overcome by novel pharmacology. Endogenous leptin fails when impaired transporters develop in obesity. The drug leptin has been modified with amphiphilic block copolymers: ethylene and propylene oxides. An example is Pluronic P85 that readily permeates the BBB. Thus the leptin conjugate P85 effectively bypasses leptin transporters and reduces food intake in obesity. Intranasal administration (nose-to-brain) also circumvents BBB. Thus the leptin conjugate P85 effectively bypasses barriers: BBB abluminal and BCSFB apical. Combined, these extrusion systems normally remove Aβ sufficiently to prevent central buildup. LRP-1 modulates tight junction proteins and remodels ECF matrix.

Glucagon-like peptide improvement of barriers injured by diabetes

Diabetes mellitus injures the BBB, increasing permeability and impairing cognition. BBB damage plays a significant role in diabetes-dependent neurodegeneration, stroke, and especially the combined disorders. Emergence of the 'CNS diabetes' concept begs the question whether insulin-system malfunction alters CP glucose transport, as a possible primary disorder in brain. D-Glucose transport at the BBB supplies neurons with energy substrate whereas glucose uptake at the BCSFB supports choroid epithelial metabolism; thus, evidence is lacking that choroidal D-glucose transport, blood to CSF, impacts neuronal glucose metabolism in health or Type 3 diabetes/neurodegeneration. Still, diabetes-altered CP ion transporter expression may affect cerebral functions by modifying CSF homeostasis/dynamics.

Deficient insulin secretion destabilizes the CNS. Glucagon-like peptide-1 (GLP-1), originating in intestinal cells, reduces insulin requirement in diabetes. GLP-1 diffuses rapidly across the BBB and is of interest in satiety models (suppressed food intake) and as a neuroprotectant.

Diabetes (streptozotocin) in rats reduces hippocampal expression of BBB tight junction proteins: occludin and claudin 5. Aquaporin 4 levels also decrease. However, treatment with the GLP-1 agonist, exendin, promotes recovery of occludin and aquaporin 4 in hippocampal microvessels. Diabetic rats also suffer losses in CP levels of claudin 2 and aquaporin 1; again, exendin reverses these protein diminutions. Exendin also restores BCSFB and BBB to normal permeability in streptozotocin-induced diabetes.

GLP-1 impacts renal fluid-regulatory mechanisms by lowering Na-K-ATPase activity, and exerting anti-oxidant and anti-inflammatory effects. This prompted Botfield et al. to investigate exendin effects on CP, the so-called 'CNS kidney'. GLP-1 receptors in CP bind exendin, decreasing the Na-K-ATPase activity tied to CSF formation. Predictably, GLP-1 reduced intracranial pressure in hydrocephalic rats. GLP-1 inhibition was prevented with GLP-1 receptor antagonists. GLP-1 is in a new class of agents with remediation potential for elevated intracranial pressure.

**LRP-1, tight junctions and amyloid removal: CP-CSF vs. BBB**

A major transporter for clearing Aβ from CNS is LRP-1 (low density lipoprotein receptor-related protein 1). Aβ removal by LRP-1 occurs at the CNS-facing aspect of barriers: BBB abluminal and BCSFB apical. Combined, these extrusion systems normally remove Aβ sufficiently to prevent central buildup. LRP-1 also modulates tight junction proteins and remodels ECF matrix.

Tight junctions are vulnerable to pro-inflammatory cytokines. Intestinal-derived microbial metabolites in the circulation alter tight junctions. Propionate protects while LPS (elevated in Alzheimer's) damages tight junctions. Astragaloside protects tight junction injury from LPS, likely by anti-oxidation. Such microbial products and their antagonists (to preserve tight junctions) deserve attention for managing the gut-brain axis in neurodegenerative
diseases\textsuperscript{142}. Regulating barrier permeability (tight junctions) and Aβ transport (by LRP-1) is a dual challenge in managing neurodegeneration.

With aging and progression of late-onset Alzheimer’s disease, the BBB transporters extrude less Aβ into blood\textsuperscript{143,144}. Subsequent retention of Aβ by cerebral disease, the BBB transporters extrude less Aβ into CSF. LRP-1 dysfunction in Aβ removal, exacerbated by inflammation, is protected by exogenous anti-oxidant N-acetyl-cysteine\textsuperscript{145}. This benefit for LRP-1 may contribute to N-acetyl-cysteine as a neuroprotectant\textsuperscript{146}.

Novel regimens are sought to enhance LRP-1 transporter strength in neurodegeneration. Vitamin D upregulates LRP-1 in vivo and in vitro. Therefore vitamin D supplementation holds promise for enhancing Aβ clearance to stall Alzheimer’s progression\textsuperscript{147}. LRP-1, in turn, upregulates claudin expression at barriers. MicroRNA 183 also modifies claudin expression\textsuperscript{148}. Therefore, stabilizing LRP-1 and tight junctions by modulators (and antagonists) may prove useful for regulating barrier integrity in the central and peripheral nervous systems\textsuperscript{149}.

LRP-1 in CP removes Aβ\textsuperscript{40} from ventricular CSF\textsuperscript{149}, thereby preventing CSF Aβ concentration from rising. Reduced expression of CP LRP-1 from toxicity (aluminum) elevates Aβ\textsuperscript{42} in CSF. Cognitive deficits result\textsuperscript{150}. In normal aging\textsuperscript{151} and Alzheimer’s\textsuperscript{152}, CP LRP-1 expression increases; conversely, BBB LRP-1 expression decreases. Therefore choroidal LRP-1 extrusion of Aβ is a ‘backup’ in aging and neurodegeneration\textsuperscript{153} when BBB LRP-1 extrusion of Aβ fails. Accordingly, this rescuing feature of BCSFB in aging is a reserve ‘transport buffer’ to attenuate Aβ buildup. Pharmacologic preservation of CP/BBB transporters and tight junctions in late life would almost certainly help to stabilize neuronal function.

**Conclusion**

Attention to the CP-CSF’s role in neural homeostasis and disease perturbation is steadily rising to the interest level for BBB. Unique physiologic properties of the BCSFB and CSF are continuously being uncovered. Supply to the brain of several endogenous substrates (e.g., vitamin C, folate, transthyretin), transported by BCSFB but not BBB, critically depends on a healthy CP. Transporters to maintain CSF oligopeptide concentrations and acid-base balance are part of CP (but not) BBB expression. Again, CSF constancy and thus brain ECF stability depend on bidirectional blood-CSF transport. Further exploration of homeostatic mechanisms expressed at the CP interface is highly desirable to advance neuromedicine.

Mechanistic information for CP extracellular matrix sheds light on leukocyte penetration from microvessels and tight junction regulation. Macrophages and dendritic cells accumulate in the choroidal interstitium, prior to movement into CSF. BCSFB tight junctions are vulnerable to disintegration by matrix metalloproteinase and other injury molecules upregulated in forebrain ischemia, hyperthermia, diabetes and helminthic meningitis. Depleted claudin proteins weaken the BCSFB, permitting paracellular infiltration of macromolecules into CSF. Claudin pharmacology advances will expand the therapeutic armamentarium to prevent BCSFB leakage.

Upon leaking into CSF, the leukocytes and microbial products distribute widely. CSF streams through the ventricles and SAS, where the Virchow-Robin spaces around major penetrating vessels admit CSF-borne materials into perivascular spaces. This long CSF pathway to brain begins in CP, an important therapeutic ‘access point’ to minimize immunopathologic spreading. CSF distribution is therefore a principal mediator in regulating the ‘upstream’ CP expeditiously to obtain a more favorable ‘downstream’ perivascular/glymphatic composition/distribution.

Neuropathologically, CP is both a target and a gateway for throughput of microbes and bacterial products (LPS) into CSF. LPS research on the BBB and BCSFB identifies the structural components of barrier breakdown, allowing the permeating LPS to provoke parenchymal inflammation and neurodegeneration. LPS experimentation, including transcriptomics, is generating new insights on sickness behavior, acute-phase proteins in CP-CSF, and sepsis-associated encephalopathy. Encouraging leads are identifying specific pharmacologic targets (MMP8 protease inhibitors and Nrf2 antioxidant activators) to curtail barrier damage from infective/inflammatory oxidative disorders.

Neuroinflammation induced by microbes threatens neuronal wellbeing. Undue bacterial or cytokine battering of CP destabilizes the BCSFB, leading to cerebral inflammation. Compromised BCSFB integrity (Helicobacter infection and helminthic meningitis) admits cytokines and leukocytes into CSF-brain. Cytokine spreading by CSF volume transmission activates microglia, promoting ‘sickness behavior’. A worthwhile goal is to counter low grade and virulent infections (SAE) in CNS by manipulating the BCSFB with immunopharmacology. For healing widespread neural dysfunction, it seems feasible to treat primary CP injuries resulting from microbes (trypanosomes) and autoimmune antibodies (NPSLE and NMDA). Accordingly, BCSFB pharmacotherapy has great potential for controlling neuroinflammation.

A range of disorders compromises BCSFB and BBB, but to variable degrees. Forebrain ischemia/stroke devastates hippocampal BBB and choroidal epithelium. Focal ischemia in areas remote from CSF damages the local neurovascular networks, without seriously impairing BCSFB. In other pathology, BBB incurs greater damage than CP. Advanced Alzheimer’s disease (AD) is characterized by cerebral
microvessel damage (e.g., downregulated LRP-1 and Aβ removal) but the counterpart LRP-1 expression in CP is expeditiously sustained. Elucidating the persistent LRP-1 expression in the aging/AD BCSFB may open a new vista to combat neurodegeneration.

New basic knowledge prompts fresh pharmacologic approaches to counter brain deficits. CP’s strategic position (e.g., healthy oxidative balance) in anticipation of high-risk complications. This could have salutary effects on attenuating stroke/seizure episodes. There is room for optimism that finely-controlled barrier transport and permeability will ameliorate many neurologic disorders.

Identifying specific neuropathology and regional barrier breakdowns is critical for devising neural repair regimens. Dual damage to BBB and CP may require bimodal remediation. Future pharmacology employing designer agents can target specific elements of the neurovascular unit and CP stroma/epithelium. Medicinals manufactured to reconstitute BBB breaches and shore up CP capabilities will likely improve CSF dynamics and benefit cerebral metabolism. Vulnerable patients can benefit from dietary/medical regimens to strengthen CP homeostatic reserve (e.g., healthy oxidative balance) in anticipation of high-risk complications. This could have salutary effects on attenuating stroke/seizure episodes. There is room for optimism that finely-controlled barrier transport and permeability will ameliorate many neurologic disorders.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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