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The molecular anatomy and functions of the choroid plexus in healthy and diseased brain

Ingrid Kratzer^{a,b}, Joakim Ek^c, Helen Stolp^d

^aFLUID Team, Lyon Neurosciences Research Center, INSERM U1028 CNRS UMR 5292, University Claude Bernard Lyon 1, 69008 Lyon, France

^bFriedensgasse 3, 8010 Graz, Austria, ingrid.kratzer@gmx.at, hedgi.ingrid@gmail.com

^cDepartment of Physiology at Institute of Neuroscience and Physiology, University of Gothenburg, Medicinaregatan 11, Box 432, 40530 Göteborg, Sweden,
joakim.ek@neuro.gu.se

^dDepartment of Comparative Biomedical Sciences, Royal Veterinary College, London, UK, NW0 1TU, hstolp@rvc.ac.uk

All authors equally contributed to the work, which included conceptualization, draft writing, review & editing.

Corresponding author: Dr. Ingrid Kratzer, ingrid.kratzer@gmx.at, hedgi.ingrid@gmail.com, +436644936418; back-up e-mail: hstolp@rvc.ac.uk

¹List of Abbreviations

Abstract

The choroid plexus (CP) is located in the ventricular system of the brain (one in each ventricle), and the CP epithelial cells form an important barrier between the blood and the cerebrospinal fluid (CSF). Their main function comprises CSF secretion, maintenance of brain homeostasis, signalling, and forming a neuroprotective barrier against harmful external and internal compounds. The CPs mature early and demonstrate expressional changes of barrier-specific genes and proteins related to location and developmental stage of the CP. Important proteins for the barrier function include tight junction proteins, numerous transporters and enzymes. Natural senescence leads to structural changes in the CP cells and reduced or loss of function, while further loss of CP function and changes in immune status may be relevant in neurodegenerative diseases such as Alzheimer's disease and

¹ AD: Alzheimer's disease, A β : Amyloid beta, ABC: ATP-binding cassette, ASPP2: Ankyrin repeat-, SH3 domain-, and proline-rich region-containing protein 2, BBB: Blood-brain barrier, BCSFB: Blood-CSF barrier, Bmal1: Arntl: Aryl hydrocarbon receptor nuclear translocator-like, CCR2: C-C Motif Chemokine Receptor 2, ChAT Choline acetyltransferase, Cldn: Claudin, CP: Choroid plexus, CSF: Cerebrospinal fluid, ICAM1: Intracellular adhesion molecule 1, GST: Glutathione-S-transferase, LV: Lateral ventricle, HClI: Major histocompatibility complex II, MS: Multiple Sclerosis, NLRP3: NLR Family Pyrin Domain Containing 3, NSC: Neural Stem Cells, SEZ: Subependymal zone, TJ: Tight junctions, VZ: Ventricular zone, ZO: Zonula occludens

Multiple Sclerosis. Neuroprotective genes expressed at CPs may be unexplored targets for new therapies for neurodegenerative diseases.

Key words: Choroid plexus, Development, Neuroprotection, Aging, Alzheimer's disease, Multiple Sclerosis

1. Introduction

The epithelial cells of the choroid plexus (CP) form a complex barrier between the cerebrospinal fluid (CSF) and the blood, and are located in the four ventricles of the brain, consisting of 2 lateral, the third and the fourth ventricular CPs. This article focuses on the CP, its morphology and function, as well as expressional changes during development and in aging. CSF secretion and neuroprotective function are especially related to the CP and are described in detail in this article. There is a particular focus on selected neurodegenerative diseases, which are associated with the CP in this overview. The unique location of the CP epithelial cells, which surround fenestrated vessels, their tight barrier, numerous transporters and enzymes (which are already present early in development), make the CP a sophisticated structure for controlling the brain homoeostasis. We suggest the need for more elaborate studies in relation to the CP to clarify further differences in molecular or functional activity between CP based on location, developmental changes, and the relevance of the CP in CNS diseases. Additional focus on gender differences in CP expression of proteins may result in new pharmaceutical, or more specific therapeutic targets at the CP in different CNS diseases.

2. Choroid plexus structure

The CP is a highly vascularised tissue, forming multiple villus structures that extend into the CSF within each of the brain ventricles (Figure 1A, B). In the lateral ventricles, the CP is elongated and extends from the ventricular floor, while in the third and fourth ventricles the CP has a clustered arrangement that protrudes from the ventricular roof. The basic structure of the CP is looped vessels surrounded by stromal tissue and an external epithelial layer. The stroma, which derives from the mesoderm, contains the extracellular matrix, and a resident macrophage and neural population. The CP epithelium derives from the ectoderm, and CP develop in a sequential manner through the ventricular system over development (discussed in detail below). The CP has a number of functions related to CSF secretion, maintenance of homeostasis and signalling through the brain; functions reflected in the structural specialisations of the choroidal epithelium.

The epithelial cells of the adult CP are cuboidal, with junctional proteins preventing paracellular transport primarily found at the apical connection between the lateral wall of the CP epithelial cells [1]. Cells have a clear polarity; microvilli extend the apical surface area (7-13-fold over the basal surface [2]), and transporters are differentially expressed between the basal and apical surfaces of the cells. Na^+K^+ ATPase and $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransporters and numerous K^+ and Cl^- channels, important for the ion regulation in the CSF, are localised exclusively on the apical surface, as well as a highly biased presence of the water channel AQP1. Similarly, xenobiotic transporters and metabolising enzymes are primarily localised on the apical surface of the CP epithelial cells. Conversely, ABC efflux transporters are typically localised to the basolateral surface of these cells (for in depth reviews of these molecular specialisations, see [3, 4]). The function of the CP is energy-intensive, and as a result, CP epithelial cells have a high mitochondrial content (10-15% of the cell volume [2]) compared to the already high 5-10% reported for the brain endothelial cells [5].

By contrast to the highly restricted paracellular diffusion of the epithelial cells, the looped capillaries within the stroma are highly fenestrated. A small neuronal population is present in the CP stroma, as well as macrophages, dendritic cells, T-cells, B-cells and NK cells [6]; reviewed by [4]. Stromal macrophages and Kolmer (epiplexus cells) are the resident macrophages of the CP. Kolmer cells are commonly associated with the apical surface of the CP epithelium (i.e. within the CSF) and have an ameboid shape, and a transcriptional expression resembling activated microglia. Stromal macrophages are more stellate, resembling traditional dendritic antigen presenting cells, and have an expression profile similar to perivascular and meningeal macrophages (reviewed by [7]).

2.1 Tight junctions (TJs) and subcellular specialisations

The integrity of the choroidal plexus barrier (blood-CSF barrier) is maintained through the presence of TJs, first recognized from freeze-fracture electron microscopy by [8]. Interestingly, these authors were not expecting such complex TJ strands in the CP, based on the relatively low transepithelial electrical resistance measured by [9]. From their findings of high strand complexity in epithelial tight junctions, they hypothesized that the complexity of the strands may not be the only important factor, and that some strands may actually organise into pores facilitating paracellular transport. This hypothesis was supported in the early 2000s, when the presence of claudin (CLDN)2 in the CP was recognised [10], and that stable transfection (*in vitro*) of *Cldn2* into epithelial cell with high transepithelial electrical resistance, was shown to create pores in the tight junction strands that facilitate paracellular water and ion movement across the barrier [11]. The majority of tight junction proteins, however, are associated with reducing paracellular perfusion and transepithelial electrical resistance, and many of these, including the established tight junction proteins occludin and

ZO-1 and adherens junction proteins alpha- and beta-catenin, are present in CP epithelial cell junctions [12, 13]; reviewed by [14].

The molecular composition of the TJs between CP epithelial cells is distinct from that of the blood-brain barrier; *Cldn1*, 2, -3 (highest expressed), -6, -9, -10, -11, -12, -19, -22 are all expressed in CP, with all but *Cldn10* differentially expressed in the CP compared to the brain vasculature, though *Cldn6* expression is differential only in development [15]. The role of each TJ protein is yet to be fully ascertained, but it is clear that some are more fundamental than others to the integrity of the paracellular barrier, for instance the cation-selective pore-forming CLDN2 may facilitate the secretion of CSF.

CP epithelial cells show canonical patterns of polarity proteins, with Crumbs and Par complexes present on the apical (CSF facing) membrane and the Scribble complex and Par1 on the basolateral membrane [13]. Interestingly, these authors suggest some deviations from normal apical-basal polarity patterning for LGL2, AP-1B and syntaxin 4 that may indicate unique drivers of polarity within the choroidal epithelium [13]. Unlike the aforementioned proteins, the polarity (and tumour suppressor) protein ASPP2 localises to the lateral cell-to-cell junctions. Knock-out of this protein results in delocalisation of ZO-1 from tight junctions and increased paracellular permeability [16]. Likewise, knock-out of ALIX, a protein apparent responsible for binding tight junction proteins to the actomyosin cytoskeleton, is associated with instability and increased permeability of the CP epithelial cells and junctions [17]. Together these imply a link between the polarisation of the CP cells and the integrity of tight junctions (discussed in [14]).

2.2 Transporters

Together with tight junctions reducing paracellular diffusion, a number of transporters are present in the CP epithelium (summarised in Figure 1C, D) contributing to its barrier and secretory function. These include active inward transporters of amino acids and ions, efflux transporters of (mostly) lipophilic substrates, bidirectional transporters and protein transporters (reviewed by [18]); there is also a substantial mechanism of vesicular transport across the CP epithelium. Transcriptomic studies have identified at least 47 solute transporters and 7 efflux transporters specifically expressed in the CP epithelium, including carriers for transthyretin, transferrin, and transporters for glutamate, glucose, amino acids, bicarbonate, metal ions, fatty acids and numerous ion exchangers, many of which are developmentally regulated and may also be differentially regulated between the CP within each ventricle [15, 19, 20]; reviewed by [21]. A neuroprotective system consisting of a plethora of enzymes and various transporters already exists in CP during development and will be described below in section 5.1.2.

2.3 Neural innervation

Cholinergic and sympathetic fibres, and receptors for these neurotransmitters, have been identified in numerous species [22], and references in [23]. Electrical activity of the sympathetic nerve reduced CSF production by approximately 30% [22], while intraventricular administration of the cholinergic agonist carbamylcholine chloride reduced CSF production by a further 30% on top of nerve stimulation [24]. More recently, work has identified a subpopulation of epithelial cells (within the mouse CP) that express choline acetyltransferase (ChAT), a common marker of cholinergic neurons. Nicotinic receptors (in distinction to the muscarinic-driven response identified by [24]), were also found, with different subunits of the receptors expressed in the three different CP locations[25]. Nicotinic stimulation of the CP resulted in increased intracellular calcium (in a subset of cells), increased transthyretin production in the 3rd ventricular CP and an increase in the highly abundant CP microRNA, mir-204 [25], hinting at a more nuanced neural control mechanism of CP function than was previously understood. In addition, highly branched neurons are visible within the CP from early in embryonic development (E12 in the chick) providing local innervation to the tissue in addition to the neural innervation from ganglia outside the CP [23].

3. Role of the choroid plexus in the healthy brain

3.1. CSF production and flow

CSF is produced by a mixture of specific transport of molecules from the plasma across the CP epithelial cells to the CSF (e.g. bulk flow of water, ions) and the specific production of CSF components by the CP (e.g. transthyretin). There is also a non-CP derived component of the CSF (estimated at 20%) that appears to derive at least partly as bulk flow from the brain interstitial fluid [26]. Numerous studies using classical inhibitors of ion cotransporters (e.g. ouabain, amiloride, furosemide and bumetanide, along with the carbonic anhydrase inhibitor acetazolamide) build up a picture of membrane specific ion transport all contributing to the regulation of CSF secretion. Primary drivers appear to be the basolateral Na^+ transporters, and apial K^+/Cl^- , $\text{Na}^+\text{K}^+2\text{Cl}^-$ and Na^+K^+ ATPase cotransporters, and transport of HCO_3^- across both membranes. The resulting ion gradients provide osmotic force for H_2O secretion through AQP1 channels and, potentially, paracellularly through CLDN2-mediated pores. HCO_3^- secretion is also very important for pH buffering in the CSF, though it is still unclear whether the carbonic anhydrase that drives its production is intracellular or found on the cell membranes (for substantive reviews on the subject see [3, 27]). There is still debate as to the exact contribution of many of these transporters in the regulation of CSF secretion.

For instance, knock-out of *Aqp1* in mice appears to only reduce CSF secretion by 25% [28], a finding inconsistent with the idea of AQP1 as a major driver of H₂O movement into the CSF. However, recent studies have suggested that co-transport of water through a number of transporters, including the NKCC1 transporters [29, 30], may facilitate non-osmotic dependent fluid secretion across the choroid plexus in addition to the role of AQP1 (reviewed in detail by [31]). It is unclear how the recent findings that NKCC1 can regulate intracellular Cl⁻ and water volume of the CP epithelial cells under basal conditions, but regulating influx of ions from the CSF [32] fits within this picture. What does appear to be clear, though, is the concept that the protein and ionic composition of the CSF varies substantially from that of the plasma, and appears to change in response to the homeostatic needs of the brain.

The protein component of the CSF is largely ‘blood-derived’ proteins, such as serum albumin, IgG, IgA and IgM, though at roughly 1000th of the levels in the blood (data summarised in [33]), and there is evidence that the CSF concentration of these proteins increases in line with increases in plasma concentration, though via a saturable mechanism [34]. There are also a number of proteins produced and secreted from the CSF, or other brain sources, that are at equivalent or higher levels in the CSF compared to the brain, for instance, transthyretin, prostaglandin-D-synthase, apolipoprotein E, cystatin C, neopterin; alpha1-antitrypsin, serotransferrin [35, 36]. This transfer or secretion is developmentally regulated, with higher protein content in the CSF during development than in the adult (discussed in detail below), and with distinct proteins present at each age, and some specific variations between species [34, 35, 37, 38].

The process of CSF flow has been reviewed extensively over the last few years [39-41]. While there are concerns that the necessary pressure gradients do not exist (discussed by [39]), the current consensus is that continuous CSF production drives CSF flows through the ventricular system from the lateral, to the third and then the fourth ventricle, before moving into the arachnoid space and out through the arachnoid granulations into the dural sinuses (as recently shown by [26]). The potential role of other outflow pathways including the glial lymphatics (gliolympmph) system, flow through the cribriform plate, or secretion back across the choroid plexus or blood-brain barrier, is still being explored [26, 42, 43], and is beyond the focus of this review. Local flow in the CSF appears to be supported by cilia on the ependymal cells lining the ventricles [44].

3.2. CP and CSF as a source of trophic factors and signalling molecules

Studies of the composition of the CSF have historically focused on the highly abundant plasma proteins described above, as well as key molecules such as amino acids. The contribution of low abundant or small molecules is only just being determined (partially due

to limitations of assessment methods). However, over the last 10 years a number of growth factors and chemokines have been identified within the CSF (e.g. FGF2, EGR, LIF; reviewed by [45]) suggesting a more complex role for the CSF in modifying the function of the brain. This is supported by an increasing body of evidence showing that molecules from the CSF, which were produced by the CP, regulated neurogenesis in both the developing and adult neurogenic niches. The transcription factor OTX2 regulates CP development and function, and knockout of this gene results in loss of the fourth ventricular CP and modification of CSF composition throughout the developing brain [46]. This alteration in CSF composition (which includes reduced total protein, increased WNT4 and increased TGM2), results in increased proliferation in the dorsal cortex progenitor zone at E13, but reduced proliferation at E16. Whereas, CP-produced IGF2 stimulates proliferation of progenitors at all ages, however its secretion from the CP is restricted to embryonic development (E15-21 in rat; [47]). This is a sufficiently strong regulator of neurogenesis in the developing brain that CSF without IGF2, from *lgf2* knockout mice, could reduce neurogenesis in cortical explants, and the knockout mice themselves could have a substantial reduction in brain weight and cortical growth [47]. OTX2 from the CP is also able to regulate adult neurogenesis [48], though in this case it appears to be through secretion of OTX2 from the CP to the CSF, on to the subependymal zone (SEZ), where it regulates extracellular matrix proteins and the astrocytes within this neurogenic niche. Similarly, the progenitor pool within the SEZ can be directly affected by miR-204 derived from the CP, maintaining them in a quiescent, progenitor state [49].

3.3. Sensing, homeostasis and circadian rhythm

The CP expresses a number of receptors associated with chemical sensing throughout the body (e.g. olfactory and taste receptors), which are differentially produced in males and females and in disease states (reviewed by [50]). The potential role of these in regulating CSF composition, through the modification of CP function, is only beginning to be explored. Gene expression in the CP changes markedly in response to sex hormones, particularly oestrogen [51], with gene pathways associated with chemical sensing, steroid synthesis and metabolism most strongly affected.

As well as producing, and regulating the composition of the CSF, the CPs also contribute to homeostasis and neuroprotection in other ways (see section 5 below). The CP produces large quantities of glutathione-S-transferases (GSTs), which bind to numerous chemical substrates preventing their movement through the CP epithelium and into the CSF [52]. In an enzymatic study, GST specific activity was found to be already high in the CP of lateral and fourth ventricles of newborn rats, and was higher than in the brain cortical tissue. In contrast,

GST activity in liver increased strongly in P35 animals as compared to P2 pups, indicating a relative immaturity of this organ in the rat at birth. This detoxification process is a particularly important mechanism in development, when liver activity is low, and activity in the lateral ventricle CP reciprocally high [52], a finding that is equally true for glutathione peroxidases that protect against oxidative stress [53]. The CPs prevent intracerebral distribution of deleterious xenobiotic compounds by means of a potent enzymatic barrier activity. The plexuses thereby fulfil an important neuroprotective function during development, especially when liver activity is still immature regarding its xenobiotic metabolism activity [54]. The knowledge of an early detoxification capacity of the CP is of utmost interest, especially regarding the more informed use of drugs in children.

A 24h rhythmicity in CP function was first suggested by [55], who identified higher carbonic anhydrase activity in the CP during a rat's dark cycle, though this was substantially less marked than circadian changes observed in the pineal gland [55]. Subsequently, a transcriptomics study of the CP showed expression of a number of genes responsible for maintaining and integrating the mammalian circadian rhythm [56]. Male and female specific production of circadian proteins was also identified in the CP, and found to be differentially produced over a 24h hour period [57]. Interestingly, these authors also found that male gonadectomy altered expression of these genes [56], an observation of note given that gonadectomy is also associated with a loss of evening activity in males. Through observation of the key circadian gene, *Bmal1*, in a *Bmal1*:Luciferase expressing mouse, high frequency circadian oscillations have been shown in the CP epithelial cells ,suggesting it may actually regulate suprachiasmatic nucleus function by communication through the CSF [58]. The oscillatory activity driven by BMAL1 was present in newborn mice, but synchronized substantially through development [59], signifying a maturation of this circadian function.

3.4. CPs and their role in immune surveillance

Immunosurveillance is carried out in part by the CP macrophage populations, which can be subdivided into the stromal CP macrophages and the Kolmer epiplexus cells in the CP epithelium. Kolmer cells appear to be long-lived cells that expand through low rates of clonal expansion (as do microglia), while the stromal macrophage population is replaced regularly with circulating blood monocytes, in a CCR2-dependent manner - possibly facilitated by their position between the epithelial cells and the fenestrated vasculature (reviewed by [7]). These macrophage populations play a clear role in the CNS response to inflammation (see below); however, their role in homeostasis is not yet fully elucidated. Of note, the CP constitutively produces molecules required for leukocyte migration, such as ICAM1 (reviewed by [60-62]),

and there is evidence of low-level immune surveillance of the CSF and brain by lymphocytes via this route in the healthy brain [62, 63]. Recent work also suggests that the stroma of the CP is used as a proliferative niche by activated T-cells from CSF, a process facilitated by the presence of the constitutive immune cells and the fenestrated vasculature of the CP [62].

The polarity protein ASPP2 also appears to dampen inflammation in the CP under baseline conditions. Not only can ASPP2 be disrupted following systemic inflammation, but knock-out of this protein results in increased inflammation within the CP [64]. The transmembrane protein Klotho also appears to play a role in the negative regulation of this constitutive inflammatory response, possibly through suppression of calcitriol (the bioactive form of vitamin D) and the NLRP3 inflammasome [65]. Interestingly, Klotho decreases with ageing, and there is a reciprocal increase in baseline MHCII and ICAM1 presence on the apical membrane of the CP [65], suggesting the potential dysregulation of the choroidal plexus immune surveillance system in the healthy ageing and/or neurodegenerative brain. The capacity for the CP to respond to immune/inflammatory signals from both its basolateral and apical surfaces, and hence to contribute significantly in neuroinflammation and neurodegeneration, is discussed below.

4. Development of the blood-CSF barrier (BCSFB)

4.1. Development and origin of the choroid plexus

The CPs appear in the ventricles at somewhat different times, with the myelencephalic first visible followed by the telencephalic and the diencephalic CP. These processes occur between E10-E13 in mice and rats, thus just when the first neurons are born in the CNS. In man, they appear between stages 18-21 (or 6-8 postovulatory weeks) and finish their growth by about mid-gestation. They grow rapidly, and the size of the CP is relatively large, compared with the brain, at early stages of brain development. Structurally, maturation is similar in the different plexuses, with an initially somewhat unorganised pseudostratified epithelium with small or no microvilli that later develop into cuboidal epithelial cells with a rich brush-border. There is also a distinct change in nuclear shape, which is highly irregular at the pseudostratified stages. Given that this could act to increase surface area of the nuclear membrane, one could speculate that this may be reflecting a large interchange of molecules and signals between cytosol and nucleus at these early stages of epithelial development. At more mature epithelial stages the nuclear shape is smooth and rounded. Noticeably, in the antenatal plexus there is a rich build-up of glycogen in epithelial cells, which can be seen between E14 until birth in rodents. What significance this glycogen has is still not known but it may provide an energy source for the plexus or brain under anaerobic conditions. Once

mature, the epithelial cells are post-mitotic and long-lived, and renewal of cells occurs slowly from the root of plexus [66]. The SHH pathway has been implicated to play a crucial role in the expansion of the plexus, driving growth with an intra-CP signalling at least in the diencephalic mouse CP. It appears that certain progenitor domains respond to SHH produced by more mature CP epithelial cells from at least as early as E12.5 just after plexus invaginates into the fourth ventricle, and this signalling acts on CP progenitor proliferation and CP expansion throughout CP development [67]. As described above, the blood vessels in the CP are fenestrated and highly permeable. It was recently shown that beta-catenin signalling is important for the maintenance of high permeability in blood vessels of CVO organs including the CP [68]. Under normal conditions, this signalling system is kept low in choroidal endothelial cells sustaining fenestrae whereas genetic stabilisation of beta-catenin reduced number of fenestrae as well as reduced perivascular accumulation of a permeability tracer. However, since these experiments were carried out in mature mice it is unclear how important this signalling system is in vessel phenotype during CP development.

As mentioned above, the two parts of the plexus, the stroma and epithelium, are of different origin. Graft experiments in chicks showed that the stroma arise from mesenchymal cells, and the epithelium differentiates from the neuroepithelium and is destined several days before an anlage can be seen [69]. We now know of some of the molecular drivers of plexus development; in order to become choroidal epithelial cells, repression of neural differentiation is needed and other molecular drivers are engaged. It appears that the drivers for epithelial CP are multifactorial and different for each plexus; drivers include WNT, NOTCH, and BMP signalling and repressors of neuronal differentiation molecules such as HES5 and HES3 (reviewed in detail in [70, 71]).

It has long been speculated that the secretome of the plexus could encompass molecules important for brain development. Indeed, the CP secretes brain-shaping factors including trophic factors and wnt-signalling molecules, among others, which shape the brain in different ways (including effects on neurogenesis, as described above). No doubt, the knowledge of CP originating molecules, that drive brain development, is going to greatly increase in the future.

There has been some controversy about the CP as a barrier interface during early stages of development, but the majority of work favours that barrier function is established almost immediately after the CP is formed. The high concentration of proteins within the CSF in the foetus has been interpreted as a consequence of poor barrier function [72] despite limited other evidence to support this view. In contrast, a series of studies in sheep foetuses showed complex tight-junctions in the epithelium at very early stages of development [73, 74]. Low-molecular weight tracers that can be visualised at EM level show evidence that TJs of the

choroidal epithelium can impede these molecules just after CP formation [19, 75, 76]. Transcriptomic studies in non-human primates supported these results and showed BBB proteins and TJ-associated genes expressed at a very high level already at earliest foetal stages studied [77]. Discrimination for flux of different albumin species between the CSF and blood in early development [34, 78] also speaks against the interpretation of high protein levels in CSF as poor barrier function. These data underpin selective transport across the CP interface and compartmentalisation of the CSF in the embryo.

The molecular composition of the TJs of the CPs is strongly regulated, and changes during development. The main TJ protein of the CPs, CLDN1, seals the paracellular pathway of the choroidal epithelium from an early embryonic developmental age and showed continuous immunoreactivity, located at the apical part of intercellular junctions in rat and human CP [10, 15]. Complete immunoreactivity for CLDN1 was also observed in CP of non-primate foetuses (*Papio hamadryas*) with no apparent difference in the staining pattern between foetuses and adult tissue [77]. Immunoreactivity in tissue was only visible at the apical cell membrane of the epithelial cells and not in other parts of the plexus [77]. By contrast, during development, the expression of the *Cldn2* was upregulated in rat CP whereas the expression of the tightening *Cldn3* was downregulated, which might indicate developmental changes in inorganic ion transport and related CSF secretion rate at the BCSFB [15]. However, CLDN3, a unique component of the CP tight junction, has recently been shown not to essentially regulate barrier permeability, with *Cldn3* knock-out mice showing compensatory increases in *Cldn1* and *Cldn2* production and no alteration in permeability [79].

Functionally, the plexus shows an increase in secretory capacity during development in sheep and rats [80-82]. However, all these studies were undertaken in anaesthetised animals, and there is overall a lack of functional studies at these ages. Nonetheless, transcriptomic studies corroborate the idea that the CP has secretory functions in development, and CSF-production associated mRNAs are increased in CP in non-human primate [83]. Of note is that the choroidal CSF secretion rate was described to increase prominently after birth in mammals [84]; timing corresponding with the developmental increase in *Cldn2* expression. There seems to be no newer studies utilising more non-invasive techniques to study CSF flow in the developing animal or human.

Taken together, these molecular, morphological and functional studies of the CP suggest that it goes through a complex developmental process from its earliest appearance in the embryonic brain. The passive barrier functions and active (influx and efflux) secretory roles of the CP do not necessarily develop at the same time, and there is clear evidence of age-specific changes in the CP, which imply specialised function over a lifetime.

5. Choroid plexus and role in neuroprotection

As previously described, the CPs are located in the 4 ventricles of the brain and are responsible for the secretion of CSF in the vertebrate brain, maintaining a stable CNS environment. The CP-CSF system is essential for the development and maintenance of the vertebrate brain, and CSF pressure is important for normal brain development [85]. Too little CSF impairs brain growth, whereas excess CSF due to overproduction, obstructed flow or limited resorption of CSF can lead to hydrocephalus [86, 87]. Several functions of the CPs are pivotal for the integrity and homeostasis of the CNS. In addition to CSF secretion and previously described functions, CPs form a neuroprotective barrier between the blood and the CSF, and prevent harmful compounds from entering and accumulating in the CSF and the brain [88]. Another role of the CP lies in the control of immune cell trafficking between the blood and the CSF, implying a function in neuroimmune regulation [63, 70, 88]. Recent gene expression studies described the general CP secretome using the lateral ventricle CP [19, 89]; however, different expression patterns during development in the CPs of lateral and fourth ventricles, as well as regional differences between the different CPs, have been shown in other studies [15, 90]. These results indicate changing functions of CPs throughout development and depending on the location of the CPs in the ventricles.

5.1. The BCSFB as metabolic barrier: mechanisms of brain protection

A sophisticated system consisting of transport proteins and metabolizing enzymes, contributes to the neuroprotective functions of the CPs. Choroidal efflux transporters and various enzymes of metabolism (phase I and II) are already well expressed at significant levels during the perinatal period [91]. The early maturation of the CP during development and the existence of above described molecules indicate that the BCSFB plays an important role in brain protection already in the neonatal stage [88]. CSF is a rich source of proteins, lipids, hormones, cholesterol, glucose, microRNAs, and many other molecules and metabolites that affect a wide range of CNS functions. CPs are dynamic from development to adulthood, as protein secretion into CSF and ciliary motility continue to mature [92]. The neuroprotective pathways, including transporters and enzymes in the CPs, are shown in Figure 2 and are described in the following paragraphs.

5.2. TJs constitute the mechanical barrier

The CP epithelium forms the anatomical barrier between the blood and the CSF, and controls the movement of solutes between the two interfaces (as described above, and in [15]). Tight junction proteins link adjacent cells of choroidal epithelium, thereby creating a physical barrier both to the passage of ions and molecules through the paracellular pathway and to the movement of proteins and lipids between the apical and the basolateral domains

of the plasma membrane. Together with this complex physical barrier, numerous antioxidant and drug-metabolizing enzymes as well as a variety of efflux transporters contribute to the detoxifying and neuroprotective functions of the CPs.

5.3. Detoxifying and metabolizing enzymes form the enzymatic barrier

Drug-metabolizing enzymes comprise functionalization enzymes which add or transform a functional group on lipophilic compounds in order to make the resulting metabolites less active and more polar. Cytochrome P450 enzymes (CYP), especially members of the CYP1-3 families, flavin-containing monooxygenases (FMO), and epoxide hydrolases play an important role in phase I drug metabolism. They inactivate exogenous compounds, including a variety of drugs, pesticides, dietary compounds and carcinogenic substances. The drug-metabolizing system further includes conjugating enzymes of the phase II type. Those enzymes add a hydrophilic moiety such as glucuronic acid, sulphate, or glutathione to the original drug or the phase I metabolite.

Phase I drug-metabolizing enzymes

There is a marginal role for CYPs in detoxification at the CPs, which is in line with low CYP activities described earlier for CP tissue [93]. In contrast to CYPs, both FMOs and epoxide hydrolases appear to play a significant role in the detoxification process at the blood-CSF barrier. High level of gene expression of *Ephx1* was described in lateral ventricle CPs and coincides with the high mEPHX1 activity measured in rat tissue [93]. Concurring is strong immunoreactivity of the mouse CP epithelium [94] along with the RNAseq data showing highest expression of *EPHX1* gene of all Phase I enzymes in the lateral non-human primate CP [83]. *Ephx1* is well expressed throughout development, indicating enzymatic protection against carcinogenic epoxides and epoxide drug-intermediates, starting from early embryonic stage onwards. Transcripts of the most important members of the FMO family regarding detoxification of exogenous compounds, *Fmo1* and *Fmo3* [95], were both described in the lateral CPs.

Phase II drug metabolizing enzymes

The phase II drug-metabolizing system consists of mainly sulfotransferases (SULT), UDP glucuronosyltransferases (UGT), and glutathione-S-transferases (GST). These enzyme families have multiple isoforms with different substrate specificities, underlining the breadth of the detoxification process [96].

Transcripts of the *Ugt1a* subfamily were highly expressed and present throughout development to adult stage. The *Ugt1a1* isoform, which could not be explicitly distinguished in the transcriptomic analysis of lateral CPs, plays a relevant role in the hepatic clearance of

bilirubin and xenobiotics. Bilirubin-conjugation at the blood-CSF barrier could be an efficient way to prevent accumulation of bilirubin in the brain as in hyperbilirubinemia [97].

Sult1a1 gene expression in lateral CPs was enriched from E19 onwards [91] which is in line with high Sult1a1-dependent enzymatic activity, measured in CP from human foetuses of 15-20 weeks of gestation [98].

Regarding the 3 families of glutathione-S-transferases (cytosolic, microsomal and mitochondrial transferases), the alpha, mu and pi classes within the cytosolic glutathione-S-transferases are mainly relevant in drug metabolism and detoxification. A study demonstrated high glutathione-S-transferase activities in rat CPs as well as human CPs of both lateral and fourth ventricles from both foetal and adult brains [88]. The transcriptomic analysis of lateral CP demonstrated high levels of gene expression for all 3 cytosolic glutathione-S-transferase classes during the perinatal period. This is also in accordance with earlier studies which showed GST-positive cells in rat CP at embryonic day 16 [99] and with the presence of *Gstp1* in CP of the human foetus [100]. In line with this, glutathione-conjugation of rat and human CP was higher in foetus and newborn than in adult [101]. The genes for enzymes relevant for glutathione synthesis were also well expressed in lateral CP already during development. The synthesis of glutathione is described in adult CP tissue [102], and transcriptomic data suggest the same capability for CPs in early developmental stages [91].

GST activities were especially high in CPs compared to that of cerebral tissue and were also higher than the activity measured in the liver at the same age, which is the reference organ for detoxification processes [103]. Glutathione-S-transferase activity was high in both types of CPs (lateral and fourth) in newborn animals and higher than in the brain cortical tissue [52]. The activity, measured in the CP of the fourth ventricle, was twice as high as in the CP of the lateral ventricle, and remained significantly higher than in the cerebral cortex at all developmental stages [52].

Glutathione-S-transferases need to work in concert with cellular efflux systems, which export the metabolites out of the cells. A functional detoxification pathway involving glutathione-S-transferases, was demonstrated in a rat model of glutathione conjugation knock-down. This study showed that the detoxifying metabolic system prevented the penetration of blood-born reactive compounds into the CSF, and already exists in the neonate. Through live tissue fluorescence microscopy, it could be demonstrated that intracellular Glutathione (GSH)-conjugates were exported by probenecid-sensitive organic anion transporters at both the choroidal epithelium and the ependyma lining the ventricles of the brain [52]. The organic anion transporter SLC21A5 and the efflux transporters ABCC1 and ABCC4 transport GSH-conjugates and are present at the basolateral membrane of the CP epithelium [88, 97]. These 3 above-mentioned transporters are the possible suspects that participate in the

GSH-dependent detoxification pathway in the CPs and are described more detailed in section 5.4.

Antioxidant enzymes

The CP cells are exposed to different kinds of oxidative stress and have a relatively high mitochondrial activity, which is a local source of oxygen-derived species. Additionally, the choroidal cells are exposed to the reactive and potentially harmful metabolites which can form in the process of phase I oxidative and reductive metabolism and some phase II reactions. CP cells have impressive antioxidant enzymatic systems for inactivating and controlling reactive oxygen species. These include enzymes such as superoxide dismutase, which inactivates superoxide anions, catalase (CAT), which cleaves hydrogen peroxide, and glutathione peroxidases (GPX), which metabolize a broad spectrum of peroxide species. Some isoforms of the GPX enzymes need glutathione in their catalytic cycle, and therefore work together with glutathione reductase, which regenerates the reduced form of the glutathione molecule [104]. Within the 8 glutathione peroxidases (GPX1-GPX8) so far identified in mammals [105], 5 were detected in a transcriptomic analysis of rat CP, and all of them were expressed at similar or higher levels during the perinatal and adult stage [91]. High levels of antioxidant enzymatic activities of GPX was described in the lateral ventricle CPs of adult rat [106]. Hydrogen peroxide is a reactive molecule that circulates in the extracellular fluid and CSF, where its level can be increased in postnatal pathological conditions [107]. At supraphysiological concentrations, hydrogen peroxide can induce oxidative damage such as lipid peroxidation. The CPs were identified as major site of hydroperoxide inactivation in the brain during the postnatal period and identified GPXs as main responsible enzymes for this inactivation [53].

5.4. Efflux transport proteins comprise ABC transporters and the solute carrier family proteins

Efflux transporters located at the apical (CSF-side) and basolateral (blood-side) surfaces of the CP can eliminate endogenous metabolites, and xenobiotics from the CSF or prevent their entry via the CP epithelium. Efflux transporters consist of primary energy-dependent unidirectional transport pumps of the ATP-binding cassette (ABC) transporter superfamily and multispecific transporters of the solute carrier (Slc) family, e.g. SLCO1A5, SLC22A8 [108, 109], and SLC15A2 [110]. The ABC efflux transporters are located at the basolateral membrane and prevent entry of harmful toxins and pharmacologic agents, including anticancer drugs, substrate for ABCC4 [111], or antiretroviral protease inhibitors, described for ABCC1 [112]. Whereas *Abcb1* and *Abcg2* are highly expressed at the blood-brain

interface, *Abcc1* is well-described in the BCSFB [113]. In line with quantitative PCR and immunohistochemistry data from [114], who described *Abcc1* and *Abcc4* expression in foetal and postnatal rat CP, a transcriptomics study of lateral CP described high expression levels of *Abcc1* and *Abcc4* in CP throughout development [91].

Within the SLC transporter family, various isoforms of each subfamily were detected in a transcriptomic analysis of CPs. They were developmentally regulated, indicating different functional roles of different transporters at developmental stages [91]. Some of the solute carriers are bidirectional and others as unidirectional inwardly-directed transport systems. Among the various organic anion transport proteins, SLC01A5, SLC15A2 and possibly SLC22A2 are restricted to CP, in comparison to other barrier interfaces in the developing brain [115]. SLC21A5, was shown to localize at the basolateral membrane of the rat choroidal epithelium, and similar to *Abcc1* and *Abcc4*, *Slc21a5* is expressed in both lateral and fourth ventricle CPs from newborn rats, as well as in cultured choroidal epithelial cells [88]. This is in line with transcriptomics analysis of lateral CP, where *Slco1a5* in addition to *Slco1c1* were highly expressed in rat CPs, both more enriched in adult compared to neonatal stages [91]. A detailed description of solute carriers and their substrates is reviewed in [116]. Using RNA sequencing, a study showed that the foetal CP of non-human primates (*Papio hamadryas*) express many enzymes for drug metabolism as well as transporters, suggesting that from, at least, midgestation, the CP in the nonhuman primate is restrictive and express most known genes associated with barrier function and transport [77].

In summary, gene expression and high activities of antioxidant enzymes such as epoxide hydrolases and glutathione peroxidases were described in the CPs as well as the activity of drug-conjugating enzymes. Several studies suggest that ABC transporters are functional at the BCSB, but their expression is developmentally regulated. The conjugation to glutathione or glucuronic acid, followed by a basolateral efflux of the produced metabolites via specific transporters to the blood, forms an efficient enzymatic barrier to the entry of those enzyme substrates into the CSF [88].

5.5. Early-expressed transcription factors for induction of neuroprotective genes (ABC, GSTs)

Transcriptomic analysis of rat CPs of the lateral ventricle revealed the expression of various ligand- and non-ligand activated transcription factors, which are involved in regulation of drug-metabolizing enzymes and transporters. Within others the oxidative stress sensor nuclear factor erythroid 2-related factor 2 (*Nfe2l2* or *Nrf2*) was found to be well expressed during development, and suggests that the neuroprotective functions of the BCSFB can be modulated in response to oxidative stress, either occurring at early developmental or adult stage. Target genes of NRF2 include GSTs and antioxidant genes. The transcript of *NRF2*

was expressed in the lateral ventricular CP of non-human primates (*Papio hamadryas*) at high levels at all ages studied with no developmental regulation [77].

Together with the ependymal cell layer which borders the ventricles of the brain, the blood-CSF barrier formed by the CPs are key cell structures in the CNS for protecting the brain environment from various chemical classes of toxic compounds already during the postnatal period. The expression of *Nrf2* and various other transcription factors that are involved in the induction of neuroprotective genes could result in pharmacological ways to improve neuroprotection at the BCSFB in relation to perinatal injuries and environmental toxicity [91]. Different pathways and mechanisms of drug metabolism in the CPs should be of interest for future pharmacological therapies related to neurodegenerative and neurological diseases.

6. The choroid plexus in aging

6.1 Structural changes in aging choroid plexus

Many structural changes occur in the natural senescence of the CP (Figure 3). Microscopic examinations show that in aged rats (2.5 years) the height of the epithelial cells is reduced by about 15% along with about 10% reduced length of microvilli [117]. This study also reports a marked thickening of epithelial basement membrane and a more moderate increase in the endothelial basement membrane. Thus, changes occur in basement membrane proteins that may impede proteolytic degradation and membrane build-up occurs. Recently, it was also reported that in elderly people the apparent diffusion coefficients of the CP, analysed from magnetic resonance (MR) imaging, was higher in humans aged 61+ compared with a younger cohort [118].

6.2. CSF production by the aged choroid plexus

One of the main functions of the CP is to secrete CSF, which has a range of functions related to nutrient provision in the brain, but also simply works to remove brain waste products. Estimations of CSF secretion rate in aged animals corroborate, with both rat and sheep studies showing a decline between healthy young to aged animals [119]. Rat studies show only a 54% CSF secretion rate in 30-month rats compared to at 3 months of age. Studies of aging sheep have revealed a similar decline with about 50% CSF secretion rates in sheep aged 7-9 years compared with 1 years [120]. There are also a number of functional studies in humans, although with contradictory outcomes. CSF production was measured in young and aged people, with significantly lower CSF secretions rates reported in the aged group [121]. However, a study using a non-invasive MR technique showed little differences between young and aged persons [122]. An important consideration when interpreting this

data are the ventricular sizes, which increase with age due to brain shrinkage, thus estimated CSF turnover rates fall dramatically and are estimated to be 3-4 times lower in aged animals. Of note, drainage of CSF has been reported to be reduced in aged mice adding to the problem of eliminating waste products from the CNS [123]. This could impact on clearance rates for toxic substances from the brain and a reduced CSF flow would also affect drug distribution within the CNS in aged people. Studies of transporters and enzymes in the CP are also indicating loss of CSF secretion capacity with age. For instance, carbonic anhydrase, AQP1 and Na-K-ATPase have all been shown to decrease in aged rats [124]. In general, there is little evidence that barrier function of CP would be compromised by aging. The protein concentration of CSF does increase with age, but this should not necessarily be interpreted as loss of barrier function, and could be explained by a range of other factors, including a reduced secretion rate or altered transport of the specific protein in question.

There have been a few studies reporting that the CP become neuroinflammatory later in life compromising brain health. In this paradigm, the CP shifts to a pro-inflammatory state, driven by peripheral immune senescence, which in turn leads to cognitive deterioration [125]. Recently it was also stated that immune quiescence in the plexus is supported by klotho, which diminishes with age, driving neuroinflammation and cognitive decline [65].

Although the above changes in the CP that appear to occur in aged animals has led to a belief that there is a general CP failure in aged people, this belief has also been challenged. A number of studies undertaken in young, middle-aged and in elderly people have been reviewed in [126], in which CSF and plasma concentration of various substances actively transported by the CP had been measured. From these they concluded that transport of transthyretin, folate, and maintenance of acid-base balance as well as electrolytes is indicative of adequate transport function even in the senescence plexus. Many other roles of CP function are yet to be examined in normal healthy aging.

7. Changes in CP morphology and the CP role in neurodegenerative diseases

Pathologies of the human CP are not uncommon, though generally not well-studied. During development, CP pathologies include cysts, haemorrhages, diffuse villous hyperplasia and tumours and are reviewed in [70]. Structural defects due to chemical or genetic means and diffuse villous hyperplasia can lead to hydrocephalus, a condition resulting from excessive accumulation of cerebrospinal fluid within the ventricles of the brain due to CSF overproduction. This can be treated by cauterization or resection as described in [127, 128].

The CP is also implicated in protecting the brain from un-conjugated bilirubin (UCB). Under normal conditions most bilirubin is albumin bound and only a small portion of unbound bilirubin may diffuse into the brain and CSF (across either the BBB or blood-CSF barrier).

The ABC transporters, PGP (ABCB1) and MRP1 (ABCC1), have been proposed to modulate brain entry of UCB [129]. However, they do not appear to be sufficient in cases of severe jaundice in newborn infants with immature hepatic conjugating capacity, where the serum binding sites approach saturation and unbound UCB (free bilirubin) will rise dramatically. Under these conditions, the accumulation of UCB in brain can produce toxicity and lead to kernicterus, resulting in permanent brain injury. Dysfunction of CSF secretion and changes in transporter expression and function could be part of the pathological processes leading to neurodegenerative diseases.

Neurodegenerative Diseases

More studies have examined the CP in relation to neurodegenerative diseases, focusing mostly on Alzheimer's disease (AD) and Multiple sclerosis (MS) (Figure 3). Structural changes and loss of neuroprotective functions of CP are discussed in more detail in the following sections.

7.1. Choroid plexus and Alzheimer's disease

Similar to aging, AD is associated with morphological changes in the CP, with thickened basement membrane, changes in the vascular and epithelial morphology, epithelial atrophy and fibrosis within the stroma, and decreased CSF secretion and transport capacity of the CP; however, these changes tend to be more pronounced in AD [130].

With employment of the Masserman technique, the rate of CSF in elderly AD patients has been estimated to 0.20mL/h, well below normal levels [131]. Corroborating this, a recent transcriptomic study of CP genes found lower levels of CSF-production associated mRNAs (including central proteins such as Na-K-ATPase and carbonic anhydrase) in AD-patients [132].

A hallmark of AD is the build-up of amyloid-beta (A β) plaques in the brain and around meningeal vessels. AD is associated with accelerated atrophy of the CP which possibly leads to decreased A β clearance via 3 mechanisms, which include the following: CSF-mediated A β clearance, direct A β absorption, and A β chaperone and protease production [130, 133], and the capacity by the cerebrovasculature to remove A β has been shown to be reduced [131]. A β removal in brain seems dependent on several transporters including LRP1 and ABCC1 (MRP1), both of which are present in the plexus. However, while in AD-patients A β accumulates in the CP [134] and this has often been interpreted as dysfunctional A β -removal at this interface the reality of this interpretation has not yet been clarified. Although BBB transport of A β diminishes, AD-models reported that LRP1 expression was increased in the CP thus potentially could counteract the loss of function at the BBB [135].

That being said, CP dysfunction impaired beta-amyloid clearance in a triple transgenic mouse model of Alzheimer's disease. Despite increased expression of the choroidal A β transporters, a thickening of the epithelial basal membrane and greater collagen-IV deposition was observed around capillaries in the CP, probably restricting solute exchanges. There was attenuated expression of epithelial aquaporin-1 and transthyretin protein compared to Non-Tg mice [135]. The reason for CP damage and malfunction in Alzheimer's disease is not totally clear and needs to be further elucidated. Further links between CP and Alzheimer's disease were recently proposed by [136] who found correlation of lipid-deposits in the CP and Alzheimer disease which seemed to be regulated by ApoE-C1q complex formation. Contrasting somewhat, but not contradicting the above studies, data review by [126] lead to the conclusion that maintenance of at least some transport systems of the CP, such as folate and transthyretin, are adequate in the CP of Alzheimer's patients.

7.2. Choroid plexus and Multiple sclerosis

Another neurodegenerative disease where CPs seem to have a central role is multiple sclerosis. MS is an inflammatory CNS disease, which involves predominantly the CNS white matter, where autoimmune responses to myelin antigens lead to demyelinating plaques. The damage to myelin sheaths and axons in this disease appears to be mediated by autoreactive T lymphocytes [137], which migrate into the CNS via disrupted blood-brain barrier and BCSFB. In MS and in an animal model of MS, i.e. experimental autoimmune encephalitis (EAE), the cytokine and chemokine milieu changes within the CP, which may lead to immune cell activation and trafficking into the CNS. As the CP is an immune-sensing organ and a sophisticated gateway for immune-cells entry in the brain, it has been examined in detail in relation to MS, and it is suggested that immune cells extravasate into the choroidal stroma from the blood vessels, entering the CSF via the CP and then infiltrate the periventricular areas [63, 138, 139]. Under normal healthy conditions leukocyte numbers are low in the CSF with only 1-5 leukocytes/ μ L which is about 2000 times lower than in blood; thus, gaining access to CSF is "privileged" to very few of the blood leukocytes. The CSF composition of leukocytes is not a simple reflection of the blood but with relatively more lymphocytes, monocytes and dendritic cells. The most numerous CSF cell type is CD4 $^{+}$ helper T-cells where a main route of entry is through the CP [138]. We and others have shown that the CP can act as a major immune cell trafficking gateway in inflammatory conditions [140-142]. In MS patients, there is an increase in mostly CD4 $^{+}$ and CD8 $^{+}$ T-cells in CSF. Molecules favouring immune-cell entry such has VCAM were found to be upregulated in the CP of MS patients [143]. Resident stromal CP immune cells seem to be activated with presentation of Major Histocompatibility Complex, Class II, DR (HLA-DR) that could act to

engage in peripheral T-cells in the immune response [143]. Furthermore, in experimental models of MS-like states such as EAE, it was shown that T-lymphocytes gain access to the brain via the CP [139, 144]. It was also recently shown that activated T-cells introduced to the CSF will home and enter the CP and could act there to provide antigen-presentation furthering the role of the CP in neuroinflammatory states [62].

In addition to evidence of leukocyte extravasation, expression of tight junction proteins were altered in an experimental autoimmune encephalitis model, indicating that the BCSFB was disrupted [145]. Selective loss of CLDN3 was observed in the BCSFB of post-mortem brain samples from patients with MS [146]. Obvious alterations in brain barrier morphology and function as well as immune cell changes are described in different studies. The underlying mechanisms and the specific role of CP in different stages of MS need further investigation, but enhanced production of proinflammatory cytokines and ROS were described to play a role [147]. A more detailed understanding can help find more cell-subtype and disease-stage specific treatment.

While the CP seem to play a central role in immune cell trafficking in MS, other blood-brain interfaces are likely to also play a role. However, the CPs could well act as a target to more precisely modulate neuroinflammation in the context of MS. Among various existing specific therapies for MS, Dimethyl fumarate (DMF; an electrophilic compound previously called BG-12 and marketed under the name Tecfidera[®]) may act, in part, on the CP. One mechanism of action of DMF is stimulation of the nuclear factor erythroid 2-related factor 2 (NRF2) transcriptional pathway that induces anti-oxidant and anti-inflammatory phase II enzymes to prevent chronic neurodegeneration [148]. The gene for the *Nrf2* transcription factor is highly expressed in the lateral CPs [77, 91]. While a definite role of the CPs in the DMF pathway and therapy process for MS is not yet described, the potential role of the CP in this and other neuroprotective agents warrants further investigation.

7.3. Choroid plexus and other neurodegenerative diseases

The role of the CP in other neurodegenerative diseases is much less studied than in those mentioned above. ALS is another devastating neurological disease in which motor neurons die in a selective and characteristic fashion; it has been speculated that CSF flow, and thus CP is central in disease initiation and progression [149]. In the SOD1^{G93A} genetic model of ALS, evidence suggests that activating the CP and enhancing leukocyte entry through the CP, and recruitment to the spinal cord, could prolong life expectancy in these mice [150]. However, during normal disease progression they found no indication of cell recruitment by the CP. A recent study compared the CP transcriptome of AD, dementia and Huntington's patients. This showed a reasonably high proportion of overlap between gene changes between these diseases with barrier-associated genes affected in all [151]. In Parkinson

patients, higher CSF/blood albumin levels have been measured [152], but this is only weakly indicates loss of CP barrier function since albumin levels may have risen for other reasons. There are also numerous reports of CP tissue grafts leading to recovery of neurological functions in animal models of Parkinsonism at least indicating a possible role of CP in disease outcome. One phase IIb clinical study in this area has recently reported findings, though unfortunately it did not meet its primary efficacy end-points [153].

8. Conclusion

The CP matures early and is a highly dynamic organ. However, its small size relative to the vascular network in the brain has often meant that the contribution to brain function and homeostasis has been overlooked. A plethora of enzymes and transporters related to drug metabolism and neuroprotection are already present in early development. Several studies suggest that numerous transporters and enzymes are functional at the BCSB, but their expression is developmentally regulated. Recent advances in the field also point to specialisations of each CP that may underlie regional functions as yet undiscovered, and require more focused study in the future. The role of the CP in neurodegenerative disease is also in need of focused and nuanced studying; in particular, the potential to regulate inflammation and drug delivery across the CP, aside from determining the role of the CP in contributing to disease pathophysiology. Keeping this in mind, regional and development-specific pharmacological therapies involving the CP could be of future interest, especially regarding neurodegenerative and neurological diseases.

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Figure 1. Diagram of choroid plexus (CP) location, cellular structure and molecular specialisation. A) Four CP are located within the brain, one in each ventricle. B) Each CP is made up of villus, cuboidal epithelial cells, joined by tight junctions and surrounding a vascularised stroma. Two macrophage-derived populations of immune cells are associated with the CP. The CP is a primary secretory organ, and to that end it has numerous solute and water transporters, as well as efflux transporters (C, D). The expression and function of these transporters changes over development, with some genes enriched in the embryonic choroid plexus (C), and others enriched in the adult (D), reflecting the developmental specialisation of CP function. Abbreviations: Abc - ATP-binding cassette transporter, AE2 - anion exchange protein 2, AQP - Aquaporin, BC - bicarbonate, C - chloride, CAR - Carbonic Anhydrase, Cld - claudin, K - potassium; Kir - potassium inward rectifying channel, Kv - voltage gated potassium channel, Slc - solute carrier, Slco - Solute carrier organic anion, Na - sodium.

Figure 2. A scheme of the neuroprotective mechanisms at the blood-CSF barrier. Tight junctions (black) form the physical barrier to the passage of ions and molecules through the paracellular pathway. Numerous antioxidant and drug-metabolizing enzymes (blue) as well as a wide variety of efflux transporters (yellowish: solute carriers, green: Abc transporters) contribute to the detoxifying and neuroprotective functions of the CPs. Only the most known mechanisms can be found on the scheme and are described in more detail in the text.

Figure 3. Cartoon of structural and functional changes of choroid plexus function reported during normal aging and in neurodegenerative diseases. Some of the roles or changes are mostly speculative at the moment and have therefore been followed by a question mark. Many changes that occur during normal aging is also seen in neurodegenerative diseases such as Alzheimer's disease but tends to be even more severe. See text for references.

Abbreviations

AD	Alzheimer's disease
A β	Amyloid beta
ABC	ATP-binding cassette
ASPP2	Ankyrin repeat-, SH3 domain-, and proline-rich region-containing protein 2
BBB	Blood-brain barrier
BCSFB	Blood-CSF barrier
Bmal1	Arntl: Aryl hydrocarbon receptor nuclear translocator-like
CCR2	C-C Motif Chemokine Receptor 2
ChAT	Choline acetyltransferase
Cldn	Claudin
CP	Choroid plexus
CSF	Cerebrospinal fluid
EAE	Experimental autoimmune encephalomyelitis
HLA-DR	Major Histocompatibility Complex, Class II, DR
ICAM1	Intracellular adhesion molecule 1
GST	Glutathione-S-transferase
LV	Lateral ventricle
MHCII	Major histocompatibility complex II
MS	Multiple Sclerosis
NLRP3	NLR Family Pyrin Domain Containing 3
NSC	Neural Stem Cells
SEZ	Subependymal zone
TJ	Tight junctions
VZ	Ventricular zone
ZO	Zonula occludens

Highlights

- Choroid plexuses (CP) make up the Blood-CSF barrier
- CP functions include CSF secretion and maintenance of brain homeostasis
- CP show developmental and regional changes of barrier-specific gene expression
- Tight junctions, transporters and enzymes contribute to a complex barrier system
- CP function in development, aging and neurodegenerative diseases (AD, MS)