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Choroid plexus function in neurological homeostasis and disorders: The awakening of the circadian clocks and orexins

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Abstract

As research regarding the role of circadian rhythms, sleep, and the orexinergic system in neurodegenerative diseases is growing, it is surprising that the choroid plexus (CP) remains underappreciated in this realm. Despite its extensive role in the regulation of circadian rhythms and orexinergic signalling, as well as acting as the primary conduit between cerebrospinal fluid (CSF) and the circulatory system, providing a mechanism by which toxic waste molecules can be removed from the brain, the CP has been largely unexplored in neurodegeneration. In this review, we explore the role of the CP in maintaining brain homeostasis and circadian rhythms, regulating CSF dynamics, and how these functions change across the lifespan, from development to senescence. In addition, we examine the relationship between the CP, orexinergic signalling, and the glymphatic system, highlighting gaps in the literature and areas that require immediate exploration. Finally, we assess current knowledge, including possible therapeutic strategies, regarding the role of the CP in neurological disorders, such as traumatic brain injury, migraine, Alzheimer's disease, and multiple sclerosis.

Keywords

Glymphatic system, sleep-wake, CSF, aging, neurodegeneration, hypocretin

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Introduction

The mammalian choroid plexus (CP) is a highly vascularised secretory tissue found within the four ventricles of the brain. Its primary functions were traditionally viewed as being limited to 1) the production of cerebrospinal fluid (CSF), thus allowing a protective buoyant suspension of the brain and spinal cord, and 2) the formation of the blood-CSF barrier (BCSFB).[1,2] However, in recent decades, additional CP functions have been identified to include 3) removal of toxic waste and metabolites from the central nervous system (CNS), 4) active contribution in the development and regeneration of the CNS, 5) immunosurveillance of the brain, and 6) regulation of CSF composition for homeostatic brain function.[3,4]

As our understanding of the CP continues to advance, it is increasingly apparent that even the slightest deviations in normal CP physiology can interfere with CNS development and function. This becomes evident in the vast number of neurological conditions to which CP pathology has been linked,[1] corroborating the role for healthy CP activity in normal CNS homeostasis. Moreover, the CP has recently been implicated in the regulation of circadian rhythmicity[5] and therefore, it is now recognized that the functions of the CP cannot be limited to those listed above. Given the strong circadian influence over the sleep-wake cycle and orexinergic signalling, a role for the CP in modulating orexinergic signalling via circadian influence is worth speculative attention. Furthermore, considering that the sleep-wake cycle modulates additional

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homeostatic processes such as amyloid-β and glymphatic clearance,[6,7] the CP may also have a significant influence on the activity of these functions.

Therefore, this review will begin with an overview of the CP and its established functions before moving on to examine the evidence for CP participation in the aforementioned novel functions relating to circadian rhythmicity. Collectively, this discussion will encourage further insight into how CP activity may contribute to the vast array of CNS processes under circadian influence. Moreover, this review will highlight ways in which this CP-circadian clock communication can be manipulated to promote repair and recovery in the stressed or injured CNS.

Choroid plexus anatomy

Three regions accommodate CP tissue: the floor of the lateral ventricles and the roof of the third and fourth ventricles.[2,8] Closely packed microvilli cover the apical surface of CP epithelial cells, which increases the surface area connecting the CP and intraventricular space.[2,8–10] Similarly, the basolateral surface consists of infoldings that increase the surface area between the ultrafiltrate in the interstitium and the epithelial cells, which enhances the efficiency of CSF production.[11] CP tissue is highly vascularized and comprises a core of connective tissue and fenestrated capillaries surrounded by a cuboidal monolayer of epithelial cells.[2,10,12–14] The large diameter of these fenestrated capillaries allow plasma to be transported rapidly via hydrostatic pressure to the CP interstitium.[10,15] The plasma is filtered by the thin membranous diaphragms that connect fenestrae, which have selective permeability for only small hydrophilic molecules and water.[9,15] The resulting ultrafiltrate is then transcellularly transported through the CP epithelium into the ventricle.[2,12,15–17] Once in the ventricular cavity, the ultrafiltrate can be utilized in the production of CSF.

In addition to its role in CSF production, the CP also functions as a selectively permeable barrier between the blood and CSF, known as the BCSFB.[4,18] The BCSFB is distinct from the blood-brain barrier (BBB), which is a tight endothelial layer between cerebral blood vessels and the interstitial fluid (ISF) that functions to restrict access to the brain parenchyma.[19] On the other hand, the BCSFB is comprised of the choroidal epithelium and the adjacent tight junctions. Three main components contribute to the functionality of the BCSFB: (1) tight junctions – inhibit solute paracellular movement; (2) selective transporters – permit transcellular movement of specific molecules; and (3) intracellular metabolizing enzymes.[2]

While this barrier's primary responsibility is to prevent the free passage of molecules between the blood and CSF, it also functions to detect and respond to changes in the blood and CSF to ensure that both maintain the appropriate chemical compositions for homeostasis.[2,17,18] Additionally, the CP plays a role in neuroimmune surveillance, as immune cell exchange between the blood and CSF is governed by the CP.[4,20,21]

Cerebral spinal fluid production and hydrodynamics

Apart from its most well-known role as the brain's protective fluid cushioning, CSF maintains a variety of other vital functions including clearing neurotoxic compounds and metabolic waste from the CNS, acting as an osmotic buffer system, and delivering instructive signals important to the regulation of CNS development and function.[18] Overall, the CSF provides the microenvironment for brain cells to survive and develop.[22]

As previously mentioned, the first step in CSF production is the passive filtration of plasma across choroidal capillaries.[2,16] This ultrafiltrate is then altered via the addition and removal of specific molecules by the CP, which allows CSF to be classified as a 'secretion'.[10,23] Since this is an active process, it requires adequate mitochondrial ATP input. The transcellular transport of water through aquaporin-1 (AQP1) into the ventricles for use in CSF production is driven by the osmotic gradient created by ion movement.[11,12,16] The brush border of the CP contains reabsorptive transporters that enable the absorption of solutes from the CSF, including glucose, amino acids, iodide, and K^{+2}.[11,23] This allows the CSF to maintain a lower solute concentration compared to the plasma, given that CSF is a dilute medium made up of ~99% water and only ~1% solutes.[12] However, ion influx from the CP into the ventricles largely exceeds the ion efflux from the ventricles, which promotes production of CSF.[2,8] As CSF travels through the CNS, its solute composition is continuously modified via exchange with the ISF.[24,25]

Both AQP1 and aquaporin-4 (AQP4) play important roles in CSF formation. While AQP1 is specific to and highly expressed on the CP epithelium, AQP4 is expressed on the paravascular astrocytic end feet, where it facilitates water movement between the parenchyma and cerebral vasculature.[2,13,26,27] Studies have shown that the genetic knockout of AQP1 results in a ~25% reduction in CSF formation,[13] however, other literature suggests that water influx for CSF is primarily controlled by AQP4, making it more critical to CSF production than AQP1.[26]

In 24 hours, the human CP generates approximately 500–600 mL of CSF, which accommodates 3–4 replacements of the total CSF volume (~150 mL).[2,11,14,16,23] The total CSF volume in monkeys, rats, and mice is ~13 ml, ~321ul, and ~35ul, respectively, while the turnover rates for these species are ~5.2 hours, ~2.1 hours, and ~1.8 hours, respectively.[28,29] Thus, CSF production rates in monkeys, rats, and mice are ~41 ul/min, ~2.84ul/min, ~0.32 ul/min, respectively. It is important to note that total CSF volume, CSF production rate, and turnover rates are dependent on many factors, including age and sex. Although the CP is the predominant contributor to total CSF volume, extra-choroidal sources account for 10–30% of CSF volume.[10,13,14,25,30]

The CSF circulation pathway begins in the lateral ventricles from which it flows through the *foramina of Munro* into the third ventricle, and subsequently through the cerebral aqueduct (also known as the *aqueduct of Sylvius*) into the fourth ventricle.[2] From the fourth ventricle, the CSF can either flow downward through the spinal cord central canal, laterally through the *Foramina of Luschka* or medially through the *Foramen of Magendie* into the subarachnoid space surrounding the brain and spinal cord. The CSF circulates throughout the subarachnoid space and is either absorbed by arachnoid granulations and transported to the superior sagittal sinus or transferred to the lymphatics.[2,11,12,15]

The choroid plexus in CNS development

There is increasing evidence to suggest an active role for the CP in the regulation of normal brain development.[3,15] Normal brain growth requires the strict regulation of CSF pressure through the production and secretion of CSF by the CP. Deficits in CSF pressure caused by CSF drainage result in abnormalities to the developing brain[31] and similarly, hydrocephalus can result from excessive ventricular CSF pressure due to overproduction of CSF or poor absorption into blood vessels.[32]

The CP also expresses and secretes a variety of proteins into the CSF during the embryonic and early postnatal periods which contribute to normal brain development.[3] Indeed, peaks in total CSF protein concentration during crucial periods of brain development in utero and near birth have been attributed to CP epithelial cell secretion.[33,34] Moreover, several proteins known to influence neurogenesis are expressed and secreted by the embryonic CP.[3,35] Across species, the developing CP expresses and secretes insulin-like growth factor 2 (IGF2), transforming growth factor–beta (TGF-β) and its subfamily bone morphogenetic protein (BMP) into CSF.[36,37] Embryonic CSF was found to regulate the proliferation and survival of cortical progenitor cells in a CSF – IGF2-dependent manner.[36] Accordingly, IGF2-deficient mouse pups show impaired cortical neurogenesis and reduced brain weights compared to controls.[36] Interference in TGF-β signalling in chick embryos suggests a role for TGF-β in the organisation and specification of neural progenitor cells.[37] BMP7-deficient embryonic cortical cells demonstrate a 50% reduction in neural progenitor cell proliferation and survival.[38] Similarly, BMP7-deficient mouse embryos display a thinner cortical layer and impaired cortical neurogenesis. Collectively, these findings reinforce the capacity for the CP-CSF system to modulate brain development.

Sonic hedgehog (Shh) is secreted into the CSF by the fourth ventricle CP to signal CP development in an autocrine manner.[39] Interestingly, the CP also contributes to the proliferation of cerebellar granule precursors via regulation of CSF-Shh concentration.[40] Cerebellar development is also modulated via CP regulation of retinoic acid (RA).[41] The developing cerebellum is highly responsive to RA. Importantly, the fourth ventricle CP, which remains closely situated to the cerebellum, shows robust RA-synthesising activity.[41,42] In the rat CP, RA-synthesising enzymatic activity peaks during periods of critical neurite branching and differentiation of deep cerebellar neurons, Purkinje cells, and cerebellar granule cells.[41] Furthermore, *in vitro* analysis supports that CP-RA activity is necessary for neurite growth in cerebellar cultures.[41] Together, these findings indicate a capacity for the CP to mediate cerebellar development. Overall, there is extensive evidence to demonstrate that the CP is crucial to normal brain development, not merely via CSF pressure regulation, but additionally via delivery of signalling molecules to developing cells and regions.

The choroid plexus in the aging brain

Several significant changes to the senescent CP have been established.[1,3] Morphologically, human CP epithelial cells become flattened with age, losing approximately 11% of their height.[43] Aged CP epithelial cells also show an increase in unique pathological protein deposits called Biondi ring tangles.[44,45] Both preclinical and clinical studies have demonstrated that the epithelial basement membrane thickens and that collagen fibres and dystrophic calcifications emerge within the stroma.[43,46,47] Exacerbating these morphological changes are additional metabolic changes that occur in the senescent CP. Enzymes required for glucose metabolism diminish by up to 26% in the aged rat CP, thereby reducing glucose metabolism and hence, energy production.[48,49] Moreover, elevated toxic products stemming from DNA damage, and the

accumulation of lipofuscin, a product of lipid peroxidation, indicate an increase in oxidative stress within the aging CP.[43,50]

Considering these morphological and metabolic changes, altered CP function is expected with age. Indeed, atrophy of CP epithelial cells alongside its reduced energy output may contribute to the reduction in CSF production and secretion seen in humans and animal models.[1,51] As a result of brain atrophy associated with healthy aging, total cranial and ventricular CSF volume doubles.[51–53] This increase in CSF volume coupled with the reduction in CSF production and secretion slows the turnover rate of CSF by three to four-fold.[28,54] While the effects of these changes on brain homeostasis have not been directly studied, several adverse implications are assumed.[49] Firstly, the efficiency of the CP-CSF system in distributing nutritive and trophic substances becomes compromised.[1,51] Secondly, diminished CSF turnover leads to reduced clearance of waste and toxins including amyloid peptides, lactate and exogenous drugs.[51] These consequences likely present as additional cellular and metabolic stressors to the brain, which may impair its ability to resist subsequent toxic insults and thereby drive age-related cognitive and motor decline.[1,49,51]

Circadian rhythms and sex differences in the choroid plexus

Of recent attention is the influence of the CP on the circadian clock of the brain.[22,55] It is widely understood that the suprachiasmatic nucleus (SCN) serves as the master circadian clock which entrains the circadian rhythms in the rest of the body.[56] However, the expression of clock genes and proteins necessary for the regulation of circadian rhythms were recently identified in the rat CP [57] and interestingly, the expression of these genes, including *Per2* and *Bmal1*, displayed strong endogenous circadian rhythms.[5,55,57] This observed circadian rhythmicity of the CP was stronger than that of the SCN and furthermore, it was demonstrated to influence the SCN clock *in vitro* and *in vivo*, likely via factors secreted into the CSF.[5] Therefore, it has been proposed that the circadian clock of the body is entrained by signals integrated from various circadian clocks, including those of the SCN and CP.[5,55]

Interestingly, CP calcifications have been associated with calcifications of other structures involved in circadian rhythmicity, such as the pineal gland.[58,59] Despite inconsistent reports regarding whether there is direct participation of the pineal gland in active generation of circadian rhythms in rodents,[60,61] it is understood that the SCN is highly sensitive to the pineal gland's release of melatonin into CSF.[62,63] Since the pineal gland is partially located within the third ventricle, pineal melatonin is directly secreted into the third ventricle and subsequently found at high concentrations in CSF.[64,65] Thus, it is worth considering whether pineal melatonin influences CP circadian clock function via control over the SCN circadian clock. Of additional importance is whether under pathological conditions, pineal gland calcifications associated with CP calcifications may interrupt CP function via altered melatonin release.

While few studies have directly investigated the effects of sex hormones on CP function, there is robust evidence to support this relationship especially given the expression of receptors for androgens, estrogens, and progesterone in the CP.[66] Unsurprisingly, the circadian rhythmicity of CP clock genes differs between males and females, with female rats displaying more distinct diurnal variations than males.[57] Indeed, estradiol influences the expression of clock genes, an effect modulated by the estrogen receptor.[67] Additional sex differences in CP function have been suggested.[66] Some of these include differences in the protein and hormone composition of CSF, BCSFB function, immune function, and toxic waste clearance.[66] Given the potential for these functions to influence normal brain homeostasis, it would be advantageous for future studies to explore the influence of sex hormones on CP function in further detail.

The choroid plexus and the orexinergic system

Sleep behaviour is governed by two principles: the homeostatic drive and the circadian system.[68] It is well-established that the SCN, situated in the basal hypothalamus, functions to maintain synchronicity between bodily rhythms and the external environment's light-dark cycle.[22] However, there are peripheral circadian clocks whose circadian gene expression phases don't align with those of the SCN, suggesting that the SCN is a coordinator, instead of originator, of circadian rhythms.[5] The CP has been shown to not only be an important peripheral circadian clock, but also the strongest circadian clock in the CNS.[5,22,57,67] Accordingly, CSF production and composition fluctuate in response to circadian rhythms.[5,69] More specifically, human CSF production reaches its lowest production rate near the end of the wakeful period (~18:00) and its maximum production near the middle of the sleep period (~02:00).[69] Similarly, CSF drainage dynamics are also likely driven by circadian signalling.[5] Communication between the CP and SCN is reciprocal, whereby the CP's strong circadian clock is also able to provide feedback and influence the SCN

master clock, presumably via signalling molecules dissolved in the CSF.[5] This feedback from the CP helps to determine the oscillation frequency and rhythmicity of the SCN clock, which then controls physiological rhythms and sleep-wake cycles. Therefore, the CP's strong circadian oscillation makes it a vital component of the brain's circadian feedback system and, consequently, the behavioural circadian rhythm.

Circadian rhythms and the sleep-wake cycle are related but independent processes. While the circadian system is predominantly dependent on the SCN, the sleep-wake cycle is largely regulated by the orexinergic system. The orexinergic system consists of a precursor protein (prepro-orexin (PPO)), two neuropeptides (orexin A and B), and two G-protein coupled receptors (orexin type-1 (OX1R) and orexin type-2 (OX2R) receptors).[70,71] Orexins (also known as hypocretins) originate from neurons in the lateral hypothalamus, but these neurons project to various other brain regions.[70–74] Similarly, both orexin receptors are highly expressed and distributed in the brain, with the distribution pattern primarily corresponding to the orexin neuron projection sites.[75,76] Interestingly, a bi-directional relationship appears to exist between the SCN and orexin neurons, where the SCN has been shown to directly innervate orexin neurons in the lateral hypothalamus,[77] while also receiving input from orexin neurons and expressing orexin receptors.[78,79] Furthermore, orexin neuron activity is regulated by circadian signalling in that it increases during wakefulness and is associated with motor activity in nocturnal animals[80,81] (See Figure 1). Evidence suggests that PPO, orexin A and B, and the orexin receptors maintain a diurnal (24 hour) rhythmicity, which regulates their expression and release.[79] More specifically, hypothalamic PPO mRNA levels, extracellular orexin A levels, and CSF orexin A levels have all been shown to decrease during the inactive (light) phase and increase during the active (dark) phase in rodents.[82–86] However, lesioning the SCN has been found to abolish the orexin A concentration rhythmicity in the CSF.[87,88] Considering the circadian influence over the orexinergic system's rhythmicity and that orexin A and B are dissolved in the CSF, it is likely that the CP and its strong circadian clock hold an influential role in the orexinergic system's timing and functioning. Moreover, it's possible that dysfunction of the CP could impair orexinergic functioning, which in turn could detrimentally affect the sleep-wake cycle.

Amyloid-β is a peptide produced and secreted into the ISF by neurons in both healthy and diseased brains.[89] However, aggregation and deposition of amyloid-β engenders severely detrimental effects on neurological functioning and contributes to the pathogenesis of several neurological disorders, including Alzheimer's disease (AD).[89] Although it is commonly assumed that amyloid-β accumulation is a result of overproduction, it is likely also a result of deficient transport through

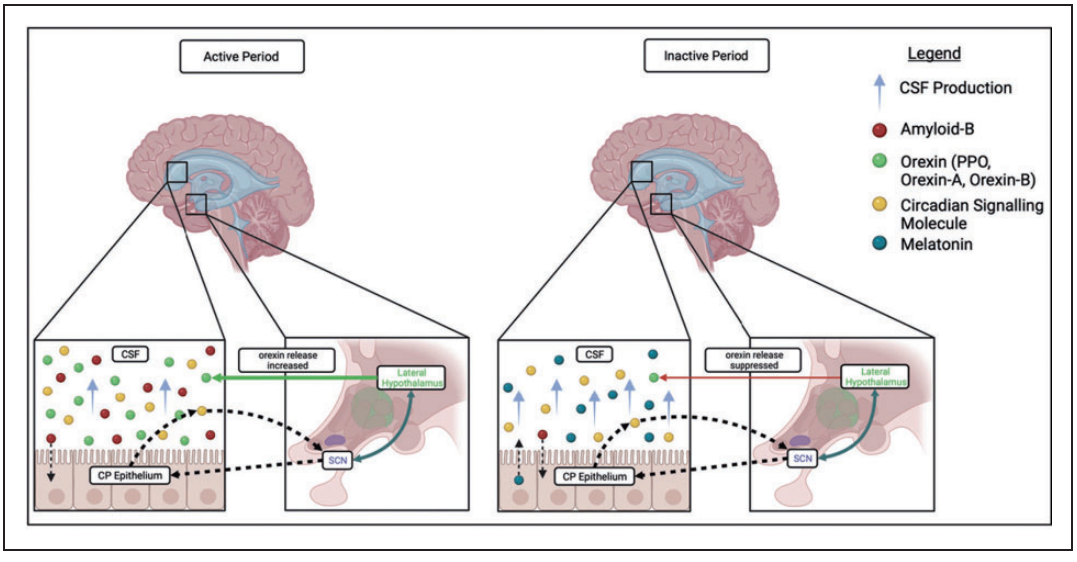

Figure 1. Several differences in CP activity and CSF composition exist between the active and inactive periods of the day. During the active period: (1) the CP reduces CSF production; (2) orexin release from the lateral hypothalamus is increased; and (3) amyloid-β levels are elevated in the CSF, since clearance is reduced. During the inactive period: (1) the CP increases CSF production; (2) release of orexin from the lateral hypothalamus is suppressed; (3) amyloid-β levels are reduced in the CSF, given that clearance is increased; and (4) melatonin is secreted by both the pineal gland and the CP. Two processes that occur during both the active and inactive periods are: (1) amyloid-β is sequestered and metabolized by the CP and (2) the CP releases circadian signalling molecules to provide feedback to the SCN.

the BCSFB or impaired metabolic clearance.[90,91] A prominent and vital function of the CP is to remove neurotoxic compounds and waste from the brain. Importantly, the CP aids in the regulation of amyloid-β levels in the CSF by rapid, non-diffusional uptake.[90] The BCSFB barrier is typically more permeable to outgoing amyloid-β, thereby supporting the uptake of amyloid-β during its efflux from the CSF to the blood. Evidence seems to indicate that, following uptake, the CP sequesters and metabolizes amyloid-β into smaller particles.[90] Additionally, the CP also secretes several proteins, including apolipoprotein J, transthyretin, and gelsolin, that support the transport and degradation of amyloid-β.[91,92] Therefore, the CP is critical for maintaining amyloid-β homeostasis in the brain and likely plays a role in the relationship between amyloid-β accumulation and the development and progression of several neurological disorders, particularly AD.

Intriguingly, the clearance of amyloid-β exhibits circadian rhythmicity and is greatly increased during sleep.[6,91] Studies in rodents have revealed that ISF amyloid-β levels oscillate in accordance with the circadian cycle, wherein the levels increase during the active (dark) phase and decrease during the inactive (light) phase.[6,91] Given that the CP is a strong circadian clock and regulator of the circadian system, it likely influences amyloid-β levels through changes in circadian signalling as well. Furthermore, disruption of the circadian clock or the CP likely causes deficiencies in amyloid-β clearance, which is a possible mechanism for the development of neurological disorders related to amyloid-β accumulation.

The diurnal fluctuation exhibited by amyloid-β in the ISF is very similar to the diurnal variation in hypothalamic orexin release.[7,86] Similar to orexin, amyloid-β levels in rodents increase during the active (dark) period and decrease during the inactive (light) period.[7] This finding has been corroborated by human studies as well, wherein human CSF amyloid-β levels increase throughout the wakeful period, peak at the end of the wakeful period, and decrease over the sleep period.[7] Evidently, the sleep-wake cycle and the orexinergic system have been shown to play a significant role in the regulation of amyloid-β levels in the brain. Amyloid-β levels in the ISF positively correlate with the length of time spent awake and negatively correlate with the length of time spent asleep, particularly with time spent in non-REM sleep.[7] Since diurnal fluctuations of amyloid-β in the ISF still occur under constant light conditions, it is concluded that these fluctuations are driven by the sleep-wake cycle and not by light or dark external cues. This is further supported by the finding that sleep deprivation significantly increases amyloid-β levels in the ISF.[7] After sleep deprivation, the following sleep period is extended and results in an immediate decrease in ISF amyloid-β levels. Similarly, chronically sleep-restricted rodents exhibit markedly increased amyloid-β plaque deposition in comparison to their control counterparts.[7] This evidence indicates that the state of wakefulness is driving the increase in amyloid-β levels, which may be due to the fact that wakefulness is associated with greater synaptic activity and that amyloid-β is a waste product created and secreted by active neurons.[93–95] Since orexin plays a critical role in promoting wakefulness and regulating the sleep-wake cycle, it is likely that orexin significantly influences amyloid-β levels. This is validated by the finding that ISF amyloid-β levels are significantly increased during orexin infusion in rodents.[7] Additionally, administration of a dual orexin receptor antagonist, almorexant, for 24 hours was found to suppress amyloid-β levels in the ISF and abolish its natural diurnal rhythm. Furthermore, chronic almorexant treatment has been shown to significantly reduce amyloid-β plaque formation.[7] Yet, termination of almorexant treatment led to immediate restoration of the natural diurnal fluctuation in ISF amyloid-β levels. Therefore, it is evident that normal orexin signalling is required for the natural diurnal rhythmicity of amyloid-β levels.

It's possible that modulation of the CP circadian clock and/or the orexinergic system could aid in enhancing amyloid-β clearance and synchronizing the sleep-wake cycle with circadian rhythms. This, in turn, could lead to critical reductions in or prevention of pathological processes that incite neurodegeneration.

The choroid plexus and the glymphatic system

The glymphatic system is proposed to be a brain-wide fluid transport pathway that functions as the macroscopic waste clearance system for the CNS.[96] This system's functional likeness to the lymphatic system and reliance on glial water flux are depicted by the term 'glymphatic'. The primary functions of the glymphatic system are to eliminate metabolic waste from the CNS and distribute compounds important for normal brain function throughout the CNS. Importantly, the glymphatic system is thought to be responsible for the removal of neurotoxic compounds, such as amyloid-β and tau, from the brain.[6,96] The glymphatic system mechanism involves the exchange of para-arterial CSF and paravenous ISF via glial AQP4 water channels located on paravascular astrocytic end feet. Glymphatic system functionality is thought to be greatly dependent on AQP4 channels, as one study found that AQP4 gene deletion reduced CSF influx and

glymphatic clearance.[96] However, the importance of AQP4 in glymphatic clearance is still controversial (Reviewed in[97]).

Evidence suggests that the glymphatic system is remarkably more active and efficient during periods of sleep while being largely disengaged during wakefulness.[6] During sleep, the brain's extracellular space expands, due to a decrease in norepinephrine, which reduces resistance to fluid flow.[98] This allows CSF to infiltrate paravascular spaces to a greater extent, thereby increasing interstitial solute clearance.[98,99] This indicates that the sleep cycle creates an environment that allows the glymphatic system to function more effectively to eliminate metabolic waste from the CNS that was generated during wakefulness.[100] Furthermore, the sleep-wake cycle and homeostatic mechanism appear to be more influential in the regulation of glymphatic activity than the circadian system, given that the glymphatic system demonstrates increased activity during both natural and anesthetized sleep.[6] Considering this, disruptions to the sleep cycle would functionally impair glymphatic clearance, resulting in inadequate waste removal and possible accumulation of neurotoxic compounds, such as amyloid-β.[6,97] This coincides with the finding mentioned previously that sleep deprivation significantly elevates amyloid-β levels and chronic sleep restriction increases amyloid-β plaque deposition.[7] In addition, peak glymphatic clearance rates occur during non-REM sleep, which is also when ISF amyloid-β levels undergo their greatest reductions.[7,98,99] This implies that non-REM sleep is the most critical sleep stage for efficient glymphatic clearance. Furthermore, given that the orexinergic system maintains such a prominent role in the regulation of both the sleep-wake cycle and amyloid-β dynamics, it seems likely that the orexinergic system would also influence glymphatic function.[97]

Although the CP produces and regulates one of the main components of the glymphatic system (i.e., CSF), it has been largely overlooked in research regarding the glymphatic system. Since the CP predominantly controls CSF production and composition, it undoubtedly maintains a regulatory role over the glymphatic system. Interestingly, CSF production rates seem to generally coincide with glymphatic clearance rates, wherein both reach their peaks during the sleep period.[6,69] Given the CP's role in chemical and immune surveillance, it is possible that the CP would respond to elevated waste product concentrations by increasing CSF production to improve waste clearance.[101] Furthermore, synchronization between CSF production and drainage is necessary for effective waste removal, meaning that the sleep-dependent activation of glymphatic clearance is most efficient when it coincides with circadian CSF production.[22] See Figure 2 for illustrative overview of relationship between the CP, BCSFB, BBB, and glymphatic system.

Overall, it appears that while the CP likely regulates glymphatic function, it also is essentially one of the main components of the glymphatic system. Moreover, impairments in CP functioning could directly affect the glymphatic system's performance, which would subsequently have important implications in the neuropathological processes driving neurodegenerative diseases. Despite being clearly connected, literature investigating the relationship between the CP and glymphatic system is exceptionally scarce and, thus, demands further attention.

Role of the choroid plexus in neurological disorders

As the CP is involved in immunosurveillance and acts as the primary barrier between the blood and CSF, it has been implicated in many neurological disorders, most notably, traumatic brain injury (TBI), migraine, multiple sclerosis (MS), and AD. Moreover, given that the CP may serve as an access point for lymphocytes to enter the CSF, allowing them to regularly surveille the brain and scavenge antigens, pathological inflammation of the CP has been suggested to precede demyelination and disease onset in these disorders.[102,103]

With respect to TBI, the CP is particularly vulnerable as the force of the injury itself disrupts the structural integrity and functioning of the CP. First, structural damage inflicted on the CP causes the BCSFB to become more permeable, which allows normally restricted cytokines, excitatory amino acids, and proteins to enter the CSF and disturb the ion/water distribution.[104,105] Since ion/water distribution largely drives the production of CSF, changes to the ion/water balance would significantly alter CSF formation and hydrodynamics. Second, leukocyte transport through the CP into the CSF is enhanced, potentially exacerbating TBI-induced edema and impeding recovery.[104,106] Third, fluid retention is a consistent outcome of TBI, which results in altered CSF dynamics and increased intracranial pressure. Finally, the waste clearance and nutritional/hormonal delivery functions of the CSF experience reduced efficiency, which is conceivably a consequence of the altered CSF dynamics.[104] These outcomes are of particular importance given that TBI contaminates the CNS with cellular breakdown products that have the potential to be neurotoxic. Therefore, the inefficient waste clearance function of the CSF following TBI likely leads to a buildup of potentially neurotoxic waste, resulting in cognitive performance deficits.[107] Similarly, efficient delivery of nutritional factors and hormones is important for

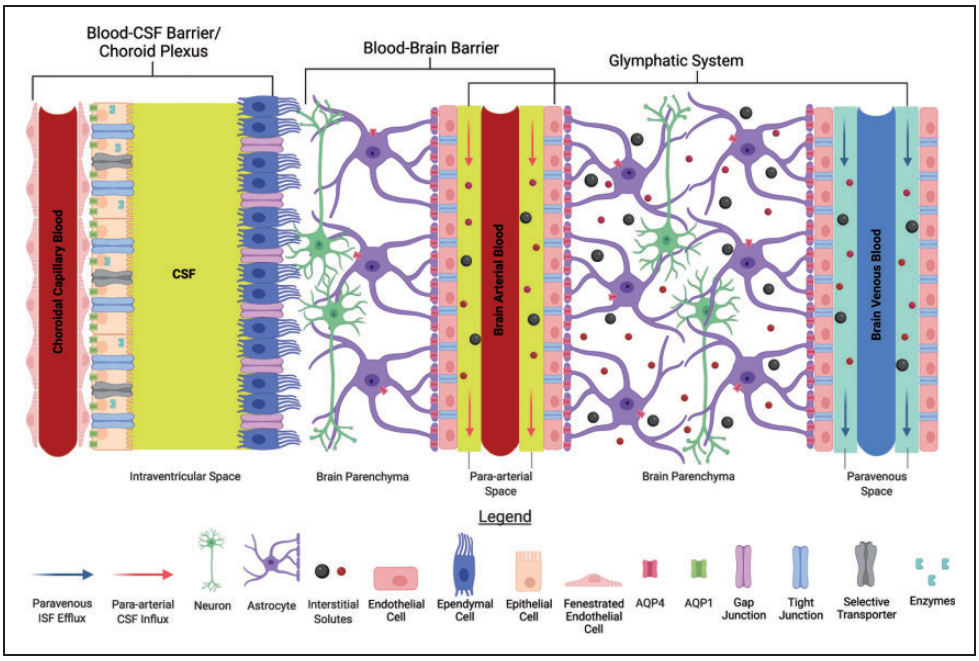

Figure 2. Depiction of the structural relationship between the blood-CSF barrier (BCSFB)/choroid plexus (CP), blood-brain barrier (BBB), and glymphatic system. (Left) The CP functions as the selectively permeable barrier between the blood and CSF, known as the BCSFB. The BCSFB is comprised of the choroidal epithelium and a fenestrated endothelium. The choroidal epithelium accommodates tight junctions, selective transporters, and intracellular metabolizing enzymes, all of which contribute to the selective permeability of the BCSFB. The ependymal layer lines the ventricle and contains gap junctions instead of tight junctions, which makes it much more permeable than the choroidal epithelium. (Middle) The BBB functions to restrict permeability between cerebral blood vessels and brain parenchyma via tight junctions along endothelial cells. (Right) The glymphatic system mechanism involves para-arterial CSF influx, CSF-ISF exchange in the brain parenchyma primarily via AQP4 water channels on paravascular astrocytic endfeet, and paravenous ISF efflux.

normal brain function but also for repairing damage caused by TBI. Consequently, this deficiency in nutritional/hormonal delivery also likely contributes to the deficits in neurological function observed following TBI.

There are numerous idiopathic dysfunctions within the CP that may lead to the neurological complications identified in these disorders. First, CP-related neurological impairment may be associated with loss of rhythmicity and synchronicity of CP clock genes.[108] *In vitro* studies of CP cells from AD mice demonstrate that there is a connection between AD and disruption to circadian rhythms within the CP.[91,108] This may result in a disconnect between CP and glymphatic function, resulting in sub-optimal clearance from the CSF, and increased build-up of amyloid-β and tau. In support of this, Dietrich et al., (2008) found that leptin clearance across the CP was impaired by accumulating amyloid-β in AD and aging patients.[109] Moreover, mRNA of amyloid-β scavengers such as apolipoprotein J and gelsolin, in the CP exhibit age, sex, and circadian-dependent expression patterns,[91] indicating that amyloid-β degradation and clearance may be compromised as rhythmicity in CP clock genes are lost with aging.

Secondly, alterations to the structural integrity of CP cells, due to calcification, cell atrophy, or chronic inflammation, may affect the CPs ability to produce CSF, synthesize and secrete proteins, scavenge toxic molecules, or adequately maintain the BCSFB,[102,110] all of which may contribute to disease onset and progression. For example, studies have indicated that CSF production in AD patients is significantly reduced, while remaining normal in Parkinson's disease and healthy aging.[111] Reduced CSF production may perpetuate chronic inflammation as it results in reduced CSF turnover and subsequent CSF stagnation; preventing the scavenging and clearance of toxic metabolites, as well as the influx of essential substrates.[51] Similarly, MRI and PET studies in patients with MS, particularly those in a relapsing-remitting stage, demonstrate that the CP exhibits significant inflammation and volumetric enlargements when compared to healthy controls.[112] The significant inflammation within the CP of MS patients has been confirmed through neuropathological analyses demonstrating increased HLA-DR, CD3, VCAM-1, and CD138 staining[102] and RNAseq analyses exhibiting dysregulation of the hypoxia-inducible factor 1 (HIF-1) pathway.[113]

Finally, literature from the migraine field suggests that alterations to activity of the BCSFB, specifically, transport of albumin and fibrinogen as well as functionality of Na^+-K^+-ATPase pumps, in the CP may be responsible for the initiation of migraine attacks.[114,115] A plethora of research has demonstrated that increased extracellular Na^+ is associated with hyper-excitability and migraine attacks,[116,117] with differential equation modelling confirming that the most likely cause of these changes in brain tissue and CSF Na^+ concentrations are perturbations to the CP transport system.[118] Using MRI, Janamiri et al. (2018) have demonstrated that CP calcification is common in patients with migraine and aura experiences,[119] suggesting that calcification of CP cells may be responsible for the dysfunction in CP transport and disruption to CSF homeostasis.

Overall, evidence indicates that the CP may be involved in many neurodegenerative processes and increasing our understanding of these impairments may offer valuable therapeutic options. For example, given that the CP secretes many antioxidants and neuroprotective factors, such as melatonin,[120] we may be able to modulate CP activity to optimize aging processes and brain function.

Conclusions and future directions

It is clear that our understanding of the roles and functions of the CP are naive and underwhelming. This complex network of intricately organized cells, once thought to merely serve as a barrier between the blood and CSF, has now been demonstrated to play a significant role in processes as diverse as neurodevelopment and circadian rhythmicity. Its significant influence on sleep-wake cycles, and subsequent removal of neurotoxic waste, renders the CP an invaluable resource for neurological health and longevity. Although we are beginning to understand how the CP may contribute to neurological disorders such as AD and migraine, we have not explored its therapeutic potential at any meaningful level. For example, given that early in life, the CP produces many growth factors needed for neurogenesis, cell proliferation, and survival, future studies could aim to 'switch this function back on' and promote the birth of new cells or repair of damaged neurons. In addition, given the significant role of estrogen and estrogen receptors on CP function and circadian regulation, this may be contributing to the significant sexual dimorphisms identified in many of these neurological conditions, offering an additional avenue for therapeutic targeting. As we continue to unlock and understand the role that the CP and CSF plays in regulating homeostasis, circadian function, and glymphatic clearance, we will generate a more comprehensive understanding of the brain and how the intricate balance between tissue and fluid contributes to neurological health and pathology.

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