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Review

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Harnessing the circadian nature of the choroid plexus and cerebrospinal fluid

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Cerebrospinal fluid (CSF) exchanges with the central nervous system's immediate environment and interfaces with systemic circulation at the blood-CSF barrier. CSF composition reflects brain states, contributes to brain health and disease, is modulated by circadian rhythms and behaviors, and turns over multiple times per day, enabling rapid signal relay. Mechanisms of how CSF elements change over circadian time and influence function can be harnessed for diagnostic biomarkers and therapeutic intervention.

Time-of-day is a critical biological variable[1]. Dysfunction in circadian rhythms is associated with neurodegenerative diseases, including Parkinson's disease, Huntington's disease, Alzheimer's disease and frontotemporal dementia (reviewed in ref. 2), and interventions to correct circadian disruptions in these disorders are attractive ways to relieve symptoms. Further, a number of neurologic conditions co-present with circadian disruptions, including hydrocephalus, autism spectrum disorder, bipolar disorder, and schizophrenia[3–10]. Sleep disruption is integral to many of these neurologic conditions and is a diagnostic criterion for major depression, bipolar disorder, post-traumatic stress disorder, generalized anxiety, and other mood disorders[11]. Many of these neurodegenerative and neurologic disorders also show abnormal CSF components that serve as disease biomarkers[3–10]. Thus, harnessing the circadian nature of the CSF system in health and disease is an intersectional field ripe for new discoveries with the potential to inform interventions for neurologic disease.

More than a century of investigations indicate that the CSF contains biomarkers for circadian rhythmicity and can relay diurnal signals to target brain tissues. CSF production robustly varies across the 24-h light–dark cycle as measured by MRI[12]. CSF distribution between brain parenchyma interstitial fluid, ventricles, and cervical lymph nodes also varies across the 24-h day[13–15]. CSF oscillations occur during sleep and wakefulness and are driven in part by neural activity both in humans and mice[16–18]. Classic CSF transfusion studies showed that CSF can carry circadian cues for drowsiness[19,20] and satiety[21], and experimental models indicate that diffusible factors released from the suprachiasmatic nucleus (SCN) of the hypothalamus into the CSF may contribute to the circadian rhythmicity of locomotion[22–26].

The brain's SCN "master clock" is entrained by daily light–dark cycles[27]. While humans, macaques, and pigs are active during the light phase, other common laboratory animals like rodents can be diurnal, nocturnal, or crepuscular[28–30]. Under laboratory conditions, common laboratory rat strains and laboratory mice, including C57BL/6, sighted C3H (corrected *rd1* mutation and melatonin competent[31]), and CD1, are primarily active in the first half of the dark phase, and many are characterized as crepuscular. These distinct periods of activity require careful interpretation of results from such laboratory animals and indicate a need to correlate any findings from different temporal behavior patterns with diurnal animals or human samples before proceeding with therapeutic recommendations.

Importantly, daily rhythmic changes can be influenced by discrete time-of-day inputs or the interaction among these timekeeping signals. Thus, entrainment to the day includes photic entrainment (i.e., the light clock) and non-photic entrainment cues, like the molecular clock or behavioral clocks that include activity and feeding cues. The molecular clock keeps time at the cellular level and dominates in the absence of photic entrainment[30]. In mammals, the molecular clock is a negative feedback loop in which a heterodimer of the master circadian regulator BMAL1 and its partners CLOCK (or NPAS2) activate transcription of clock-controlled genes, including Period (*Per*) and Cryptochrome (*Cry*) that code for repressors of BMAL1 heterodimer activity, thus closing the loop that generates rhythms of approximately 24 h[32,33]. The light clock is the SCN of the hypothalamus. The SCN is entrained by daily light–dark cycles, and SCN output includes circuits and release of neuropeptides that communicate this light information to the rest of the body[23,24,34]. Finally, behavioral input, including locomotion, stress, and feeding, can modulate metabolism and shift or disrupt daily rhythms[35,36].

Progress toward understanding bi-directional daily CSF regulation and signaling remains an active field of study (previously reviewed in refs. 37–41). However, because of the diagnostic and treatment capacity of CSF, the goal of this review is to characterize the actions, dynamics, and composition of this biofluid as a promising way to better understand the interactions between circadian rhythms and central nervous system (CNS) function and health.

Overview of the cerebrospinal fluid (CSF) system

CSF

CSF is an aqueous solution of ions (Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-), glucose, metabolites, proteins, neurotransmitters, cytokines, nanovesicles, and

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hormones (including thyroid hormone, atrial natriuretic peptide (ANP), serotonin (5-HT), melatonin, insulin, leptin, ghrelin, and cortisol). Its traditionally assigned roles include providing a buoyant fluid cushion and pH and ion balance for the CNS. CSF also plays critical roles in regulating the CNS throughout life, including the distribution of essential health and growth-promoting factors[42–47]. It also removes waste byproducts that reflect CNS state and have, therefore, been harnessed as disease biomarkers for conditions including injury like traumatic brain injury and spinal cord injury, metastasis, degenerative diseases (amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease), neurological diseases (autism spectrum disorders, schizophrenia), and neuroinflammation. Although there is not free exchange between CSF and the interstitial fluid of the brain parenchyma in adulthood[48] or during brain development[49,50], these compartments do exchange solutes. This exchange happens in a diffusion-limited way at the ependyma ventricular surface in adult brains and along permissive routes, including perivascular spaces, white matter tracts, and subependymal spaces perivascular spaces after they mature[51]. Perivascular exchange of solutes between CSF and parenchyma is well-characterized[52–54], although directionality, especially dominant parenchymal CSF outflow routes, and the question of convective flow through the parenchyma remains under active study[51,55]. One hypothesis proposes a glymphatic system of directional CSF influx along perivascular spaces lined by astroglial endfeet with polarized aquaporin4 (Aqp4) expression, directional parenchymal convective flow, and venous clearance. The polarized Aqp4 expression is implicated as a critical component of glymphatic exchange and reduction in this expression reduces solute eliminate rates between CSF and brain parenchyma[55,56]. Why Aqp4 reduces parenchymal solute clearance remains an active field of study. Further, brain solutes that originate in parenchyma are found in lymph nodes prior to entering the blood, implicating multiple routes of parenchymal solute clearance. Continuing to generate a more complete understanding of CSF exchange with brain parenchyma and solute efflux is important to a holistic model of CSF circadian rhythmicity, as parenchymal solute clearance rates and CSF solute parenchymal influx rates do change with respect to time of day and activity[15,18,41,57,58].

Choroid plexus

Choroid plexus tissues are located in each of the four brain ventricles, or cisterns—two lateral ventricles, the 3rd ventricle, and the 4th ventricle (Fig. 1A). Transcriptomics from mouse has identified molecular differences among lateral ventricle choroid plexuses vs. 3rd ventricle choroid plexus, vs. 4th ventricle choroid plexus[59], but the overall structure is similar. Choroid plexus is a specialized tissue of neural origin whose structure includes fenestrated capillaries interfacing with a fibroblast and mesenchymal core that is surrounded by a monolayer of specialized epithelial cells with highly articulated CSF-facing apical structures, including microvilli and tight junctions at the apico-lateral interface. These tight junctions, along with polarized transporters and enzymes, form the blood-CSF barrier that actively regulates exchange between CSF and systemic circulation. The choroid plexus also contains resident immune cells, including macrophages, T cells, B cells, and Th17 cells. During immune challenge, the choroid plexus can become populated by neutrophils and other markers of inflammation[60,61], including barrier changes like closing endothelial fenestrae after inflammation in the adult gut[62]. Choroid plexus epithelial cells (Fig. 1A) exhibit a very high apical surface area for interacting with the CSF[63] and these epithelial cells are highly energetic/ metabolically active. They contain a cohort of ion and water channels[64], detoxifying transporters[65–67], and apparatus for vesicular transport and paracrine/apocrine signaling[59,68,69]. Of potential relevance for daily signaling, choroid plexus epithelial cells of the mouse lateral ventricle choroid plexus are depleted for insulin signaling compared with 3rd and 4th ventricle choroid plexus tissues, with lower expression of Ins2 than the other two tissue types[59,70,71]; and 4th ventricle choroid plexus epithelial cells express neuropeptides like endogenous opioid proenkephalin (*Penk*) that are not expressed by the lateral or 3rd ventricle choroid plexus tissues[59]. These examples of key differences among choroid plexus tissues reinforce the importance of treating each choroid plexus tissue separately when measuring diurnal changes and functions. Changes in choroid plexus epithelial cell barrier, ion transport, enzyme expression, or secretion apparatus are key elements that can change CSF composition. Thus, the choroid plexus regulates CSF composition both through its barrier and secretory roles. The choroid plexus is also innervated by the sympathetic, parasympathetic, cholinergic, and peptidergic systems, which have been shown to contribute to regulating CSF production or composition in mammalian models[72–74]. Since the autonomic nervous system coordinates circadian functions throughout the body[75], this innervation is likely upstream of some choroid plexus daily changes. Since the choroid plexus plays discrete key roles in CNS health, fluctuations in choroid plexus CSF production, solute secretion, or barrier functions are likely the most important to consider when including it in a discussion of the circadian system.

Circumventricular organs and tanycytes

Circumventricular organs are midline structures contacting the ventricular system that are open to neuro-hemal exchanges largely for endocrine purposes. They include the median eminence, organum vasculosum, laminae terminalis, subfornical organ, subcommissural organ, pineal gland, and area postrema[76]. These organs can directly impact CSF composition and even dynamics, for example subcommissural organ ependymal cell cilia motility in response to CSF glucose can alter local flow rates and secrete *Wnt5a*-positive vesicles[77] in rodents. In humans, the subcommissural organ is only present in fetuses and can be distinguished from 7 weeks to 5 months, playing the role of an active secretory structure into the CSF[77,78]. Some of the roles of the subcommisural organ in humans may be taken on by ependymal cells of the hypothalamic median eminence and choroid plexus[77]. Of key importance to the circadian system, the pineal gland is a neuroendocrine circumventricular organ where information about photoperiods and ambient temperature or food availability is transduced into the chemical signal, melatonin, which is observed in the CSF[79]. In the median eminence, tanycytes—specialized radial glial cells— line the third ventricle and regulate of broad range of hypothalamic functions[80]. The apical side of median eminence tanycytes harbors tight-junction complexes, thereby preventing the passage of blood-borne molecules into CSF. On the basal side, tanycytes contact fenestrated vessels in the median eminence and capillaries in neighboring hypothalamic nuclei[81]. Relevant to time-of-day signaling, insulin receptors in tanycytes of the mediobasal hypothalamus are necessary to regulate insulin access and control systemic insulin sensitivity[82].

CSF dynamics and outflow routes

CSF is estimated to turn over ~every 5 h in humans and ~every 2 h in mice[83–85], as calculated by total volume/production rate. But a third variable, CSF outflow, also contributes effective turnover. As CSF is produced, it moves throughout the brain ventricles, subarachnoid space, and spinal canal and is drained into the blood or extracranial lymphatic system[84]. Several putative CSF clearance routes include arachnoid villi and granulations in human (and only arachnoid villi in non-human mammalian species), perineural and perivascular pathways, and meningeal lymphatics[44,55,86,87]. It has been shown in rats, pigs, and sheep that lymphatic vessels contribute to CSF clearance by connecting with CSF around the cribriform plate and olfactory nerve roots during perinatal brain development[88–90]. Meningeal lymphatics play key roles in clearing CSF. In fact, CSF components, including cellular debris after injury, can be found in the cervical lymph nodes. Recently, roles of the pressure-sensitive Piezo1 channel have been identified to regulate meningeal lymphatic CSF clearance and the chemical agonist Yoda1 is sufficient to increase CSF outflow[91,92]. Other activities that change across the time of day including breathing, sleeping, and physical activity[93] can impact CSF mixing and outflow in humans.

Development

The CSF system functions distinctly as it matures. Since the adult mechanisms of generation and outflow only arise later in development, other processes govern this system early. CSF composition is quite distinct in early development with protein[46,47,94] and ion compositions[95–97] maturing throughout development. CSF composition governs some of the active roles of the CSF in maintaining early brain health. For example, the unique

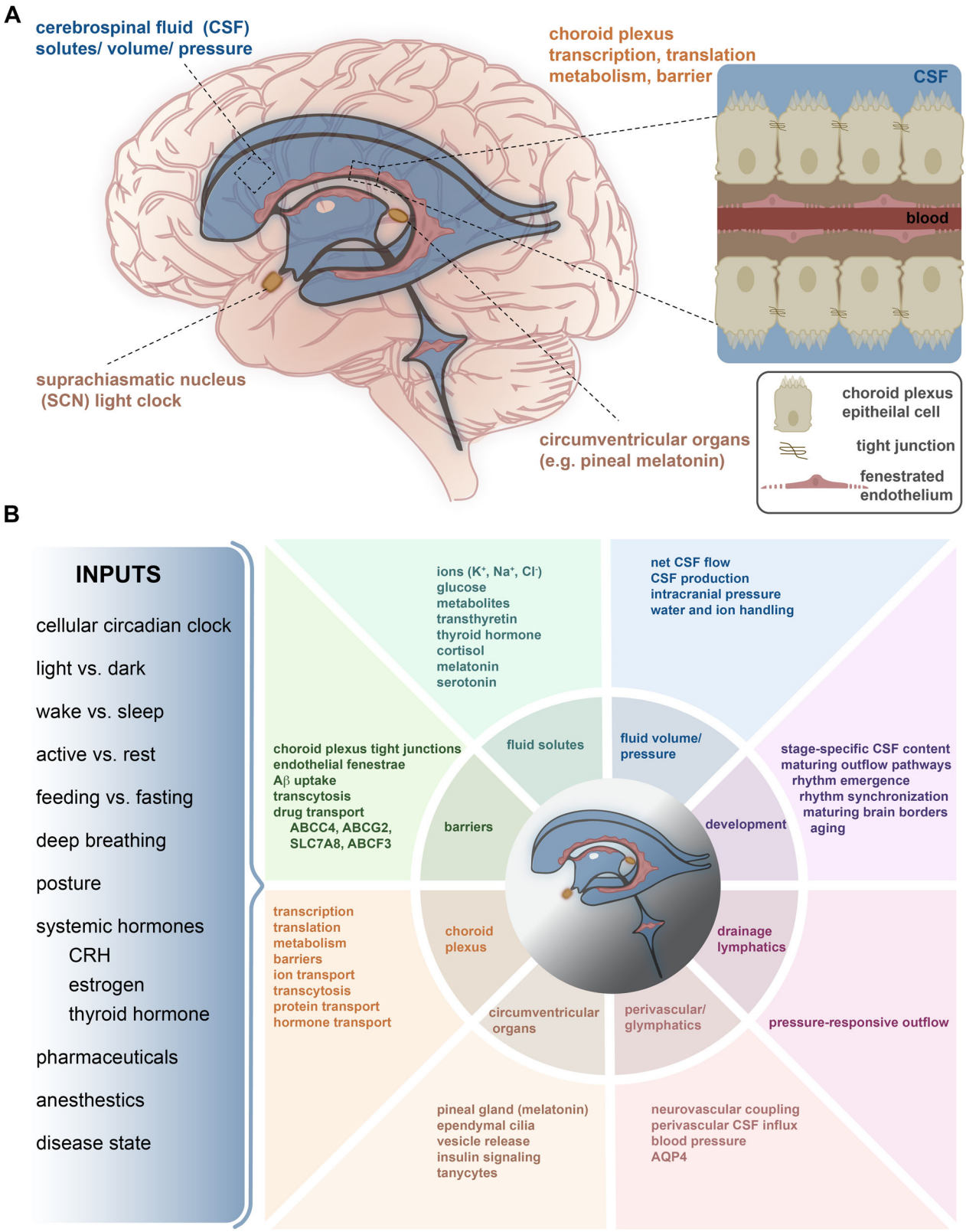

Fig. 1 | Components of the cerebrospinal fluid (CSF) system change across time of day. A The major components of the CSF system include CSF, choroid plexus, and circumventricular organs. These systems change their properties across time of day, including those listed below each component. **B** Multiple inputs affect the CSF system across time of day.

composition of proteins like growth factors and morphogens[46,47,98–102], neurotransmitters[71,103–105], nanovesicles/endosomes[106–110], non-coding RNA[106,107,111], metabolites[112], and hormones[112] confer CSF the ability to maintain developing neural progenitors. Further, CSF composition can reflect key brain developmental stages, as CSF proteins match the changing gene expression of normal early CNS parenchymal development[113,114]. The CNS developmental timeline informs which CSF components are present and how they mature as the brain develops. CSF arises after neural tube

closure (~E9 in mice), and the brain ventricles begin to form and develop. Meninges also interact with CSF and are present early (~E8 in mice) and mature later (~birth in mice)[115]. Choroid plexus begins to emerge after CSF is detectable (~E11 in mice) and continues maturing its ability to transport ions and other functions during the first postnatal weeks[44,59,96,102,116–118]. Perivascular components begin to arise with endothelial tight junctions emerging as the choroid plexus matures (~E12 in mice) and blood-brain barrier function is evident at E15.5[119]. Ependymal cells that line the mature brain ventricles are generated by radial glia beginning at E14 in mice and mature around P5 to display fully functional motile multi-cilia[120]. The arachnoid barrier is formed by E17 in mice[121], but the precise developmental functional timeline of arachnoid villi and their relative contribution to CSF outflow remains less clear. The larger arachnoid granulations are not recognized in rodents. In humans, they appear after 39 gestational weeks they are not fully formed until 2 years of age[122]. Meningeal lymphatic vessels sprout from existing vessels at the base of the skull around E18 or birth in mice, and this system continues to mature throughout the first postnatal month[115]. Maturation and polarization of perivascular astrocytes, including the hindfoot-basal lamina junctional complex and Aqp4 (implicated in the glymphatic model) occur ~P7 in mice[123], after the arachnoid barrier forms, and mature perivascular CSF infiltration and parenchymal exchange emerge by P14 in mice[124]. As natural aging proceeds, changes to CSF composition[94,125], CSF production and turnover[126], choroid plexus function[59,127], meningeal lymphatics[87], glymphatic exchange[56], and other systems begin to occur. Whether these changes with aging are affected by or impact the overall reduction in circadian regulation observed with ageing[128] remains an important open question.

Daily rhythms of CSF solutes. As highlighted above, CSF solutes are complex and dynamic (Fig. 1B). They can arise from multiple source tissues, be the result of CNS waste clearance, brain barrier function, or actively regulated transport, detoxification, or secretion processes. The production of some diurnally regulated CSF solutes cycle in their source tissues including cortisol production by peripheral adrenal glands, corticotropin-releasing hormone (CRH) release by hypothalamus and melatonin from the pineal gland[23,25,79,129–131]. The light-entrained SCN pacemaker can interact directly with the CSF through release of diffusible factors[26]. The SCN diurnally releases vasoactive intestinal peptide (VIP), arginine vasopressin (AVP), and gastrin-releasing peptide (GRP) which can be observed cycling in CSF. Lesioning the SCN abolishes the orexin A (hypocretin-1) concentration rhythmicity in CSF[132,133]. Further, the caudal dorsal raphe of the hypothalamus maintains serotonergic axons that directly reach the CSF in both nocturnal and diurnal animals[73,74]. Using a fluorescently tagged cholera toxin beta subunit retrograde tracer injected into the CSF of nocturnal and diurnal animals, the location and pattern of labeling was localized in the hypothalamus to the cluster of serotonergic neurons in the caudal dorsal raphae in both subtypes[134]. This consistency between diurnal and nocturnal model organisms suggests that it is the downstream interpretation of these dorsal raphe-to-CSF serotonergic signals that are modified for the differential needs of diurnal vs. nocturnal animals rather than this circuitry itself.

Other key CSF solutes, like thyroid hormones, show diurnal differences in CSF concentrations that correspond with systemic thyroid hormone and the expression of CSF thyroid hormone carrier protein transthyretin (TTR) in choroid plexus[135]. Modulating systemic thyroid hormone has recently been implicated in coordinating thyroid hormone gene programs that drive daily behavioral changes, including feeding and exploratory behavior[136], with the potential to directly interface with CSF thyroid hormone and thyroid hormone transport into the CSF as regulated by choroid plexus transporters and carriers[135]. Further, circadian variations in metabolism induce differential CSF metabolites[135,137]. The CSF and choroid plexus both reflect a more oxidative signature during the active phase[135]. Modulating brain interstitial ions has been shown to alter sleep-wake state[138], opening the possibility of functional roles of CSF ions throughout the day. Studies of human and animal CSF ions find that CSF K^+, Na^+, and Cl^- decrease during sleep phases[138–140] and can change during seizure activity[140]. While CSF Na^+ and Cl^- remain at intermediate concentrations during sleep deprivation, CSF K^+ is low both during healthy sleep and during wakeful sleep deprivation[139], suggesting a circadian, rather than sleep, induced modulation of CSF K^+, which may reflect any combination of choroid plexus ion modulation, neuronal activity, and glial K^+ buffering.

Because solute concentrations in fluids like CSF are dependent on dilution, there is the possibility that the observed cycling of CSF protein or ion levels is an emergent property of fluid production dynamics. However, this is not the case for all solutes because CSF solute cycling peaks and nadirs are specific to select solutes. For example, no significant circadian fluctuations were found in CSF levels of klotho, a key CSF component[141] or other CSF ions like calcium or magnesium. Thus, it is a reasonable conclusion that circadian rhythms of individual CSF solutes are actively regulated and reflect specific changes in the CNS, act as key functional signaling components of CNS circadian output, or both.

Daily rhythms of CSF volume and pressure. Daily changes in human CSF dynamics were first observed by Nilsson[12] who measured CSF production by magnetic resonance imaging of net CSF flow through the cerebral aqueduct at distinct circadian times (Fig. 1B). In this study, the average CSF production in six healthy volunteers indicated circadian variation, with minimum production at 6 p.m. of ~12 mL/h which was ~30% of the maximum observed values at a nightly 2 a.m. peak with production of ~42 mL/h. These findings were corroborated in an independent set of intensive care unit patients over 30 years later[142]. In Sprague-Dawley rats, a 30% increase in intracranial pressure was observed during the night, which is the active phase for these rodents[142]. These findings of peak CSF production/pressure at orthogonal phases of activity in humans and rats raise the possibility of independent control of CSF pressure (perhaps by light-based clocks) versus CSF metabolites, which were more alike at equivalent active phases (perhaps by feeding). Further, Sprague-Dawley rats are crepuscular rather than nocturnal, so the daily activity differences between these models and humans may further complicate the comparisons between light vs. dark phases, active vs. rest phases, or sleep vs. wake periods. This study also suggests that the daily increase in intracranial pressure is independent of vascular parameters[142] that could influence the perivascular CSF influx[57] that changes between day and night[14,15] and after sleep deprivation[18,143,144]. Together, these findings indicate that circadian rhythms of CSF volume and pressure may be at least partially independently regulated from the circadian rhythms of parenchymal CSF influx.

The mechanisms of circadian changes to CSF volume or intracranial pressure are still under investigation. Such changes could be downstream of activity-dependant buildup of solutes in the interstitial fluid that, when cleared, drives water into the CSF system through osmosis. It could also be more specifically regulated by glucocorticoids, whose levels cycle over circadian times and can increase intracranial pressure in rat models[145]. Observed changes in CSF dynamics may also be directly driven by the choroid plexus, which is specialized to secrete CSF in adults through ion transport and water secretion. The rhythmic cycling of both expression and activation of the carboxylic anhydrase (*CAII*) in the choroid plexus has been observed in rats with a drop in activity during the light phase[146], and this rhythm could alter CSF secretion. Carbonic anhydrases enzymatically convert HCO_3^- and H^+ from H_2O and CO_2, and while they are not directly involved in ion transport, they have important roles in CSF secretion as carbonic anhydrase expression is a key event in early choroid plexus differentiation[147]. While a slight rhythm for the major apical water transporter on choroid plexus epithelial cells, aquaporin1 (Aqp1), has been reported in some systems, Aqp1 is likely not robustly regulated at the circadian level in rodents[135,148]. Therefore, the major drivers of these circadian changes in CSF dynamics remain to be defined.

Daily rhythms of choroid plexus. Cycling elements of the molecular clock have been identified in all mature tissues in the body where they have been investigated[149–151], including the major CSF-producing tissue,

the choroid plexus[135,152,153]. Each molecular clock in the body responds to systemic synchronizing cues, however the peak phases of these rhythms vary among individual brain tissues and the periphery, which, in turn, differ between diurnal and nocturnal animals[154]. These circadian cycles include general and tissue-specific changes in the choroid plexus. Since the choroid plexus has some rhythmic gene expression elements throughout the day, validated stable (non-cycling) gene products like beta actin (*Actb*) and hypoxanthine-guanine phosphoribosyltransferase (*Hprt1*) represent important reference genes when normalizing circadian data in these tissues[155].

One of the earliest reports of rhythms in the choroid plexus is the observation of circannual (seasonal) rhythmicity in the antidiuretic effect of choroid plexus extracts (elevated in Sept–Dec), vesicle load (elevated in winter), and the glycogen load/mitochondria abundance (elevated in winter) in toads (*Bufo bufo*) in the U.K.[156]. Further, choroid plexus shows a striking rhythmic seasonal pattern of activation (by *c-fos* expression) in a hibernating squirrel—the thirteen-lined ground squirrel[157]. With strong *c-fos* activation reported in choroid plexus epithelial cells and 3rd ventricle tanycytes that peaks as arousal from hibernation initiates. In arousal, c-fos activity in choroid plexus epithelial cells diminishes along with diminished activity in SCN and reticular thalamic nuclei, however activity in ependymal cells increased during the arousal phase, indicating complex independent regulation of choroid plexus from other ventricular structures throughout these hibernation cycles[157].

Circadian clock gene expression in the choroid plexus was observed in a study investigating the effects of sex hormones on the choroid plexus by microarray on male and female sham vs. gonadectomized mice. This study identified significant changes in clock genes among these populations[158]. In light of these observations, circadian findings in the choroid plexus were expanded to better understand roles of sex hormones on choroid plexus rhythmicity[159], finding that choroid plexus rhythmicity is modulated by estrogens[153]. Ex vivo choroid plexus circadian rhythmicity is sensitive to exogenous application of a synthetic glucocorticoid analog (dexamethasone) both when applied ex vivo[135] or in vivo[160]. In animals lacking glucocorticoids (corticosteroids that bind to the glucocorticoid receptor) due to the removal of adrenal glands, the rhythmicity of *Per1, Per2, Nr1d1, and Bmal1* expression in choroid plexus were all dampened[161] and conversely rhythmic administration of dexamethasone reinforced these transcriptional rhythms in choroid plexus[161]. Sex hormones and glucocorticoids are broadly implicated in both choroid plexus function and CSF homeostasis[88,145,162]. Indeed, the circadian choroid plexus response to both sex hormones and glucocorticoids remains one of the more deeply studied aspects of choroid plexus circadian rhythmicity.

The developmental timing of this rhythmicity emergence in rodents was investigated using a *Per2*-Luciferase mouse[135,152,163] and in rats[162]. Stable exogenous choroid plexus *Per2* rhythmicity is evident at birth in mice, but the zenith and nadir remain desynchronized between young animals and the period is variable. *Per2* rhythmic expression synchronizes across animals to a 24-h oscillation period around P11[152]. Choroid plexus circadian rhythmicity results in substantial changes in protein translation, including secreted proteins, mitochondrial proteins, and barrier components[135]. These cycles in protein expression correlated with altered choroid plexus secretome, metabolome, and barrier structure. Diurnal rhythms in choroid plexus transcription have been observed in rats that are consistent with metabolic and secretome changes shown in mouse[137]. Further detailed analysis of coordinated choroid plexus rhythmic gene expression classified suites of gene expression with the predominance of peaks around CT17-21 and other sets of genes that peak in expression around CT8-15[160]. The core clock component *Bmal1* and feeding cues mediate choroid plexus rhythmicity[135,152], and intact SCN activity is required for choroid plexus diurnal translation responses[135] and circadian gene expression[160]. Melatonin is synthesized by porcine choroid plexus explants, although no circadian pattern has been observed in choroid plexus melatonin synthesis[164]. However, melatonin can reset the choroid plexus circadian clock in an immortalized cell line derived from primary rat choroid plexus epithelial cells (Z310 cells)[165]. In contrast, choroid plexus circadian rhythmicity has been shown to be resistant to lipopolysaccharide immune challenge, which dampens, but does not abolish, rhythmicity in the choroid plexus, unlike the strong supression of the liver circadian clock in response to immune challenge[166].

Functional assays of choroid plexus circadian roles remain an open field of study. Circadian changes in transporter function can regulate transport across an in vitro model of the choroid plexus barrier[167,168]. Co-culture of choroid plexus and SCN implicate a factor secreted by the choroid plexus that can influence SCN rhythmicity, but whose identity is not yet elucidated[152]. After removing core clock component *Bmal1* from the choroid plexus and other multiciliated cells using the Foxj1-cre mouse line, large-scale diurnal changes in choroid plexus translation were observed along with altered CSF contents and disruptions in diurnal behaviors[104]. However, in a different mouse model with a substantial reduction of the choroid plexus in adults—the ROSA diphtheria toxin receptor (DTR) mouse line—no circadian behavioral disruptions are observed[169], indicating that the behavioral disruptions observed after *Foxj1-cre* induced *Bmal1* conditional loss of function are either due to non-choroid plexus functions, differences in mouse lines, or are compensated for in the DTR model. Laboratory approaches that specifically modulate the choroid plexus in vivo to enable circadian behavioral readouts remain under development[170], but harnessing the viral tropism of AAV2/5 or developing new transgenic lines that are specific to the tissue will be crucial to advancing this field that ultimately requires integrated whole body readouts.

Implications of daily rhythms in the CSF system for chronopharmacology and disease. The blood-brain barrier and blood-CSF barrier are key considerations for successful pharmaceutical targeting to the CNS because they limit access of systemic substances to the CNS. While the blood-brain barrier has been increasingly studied, detailed knowledge of the blood-CSF barrier and the cell types involved in both of these barriers remain less-well understood (Fig. 2). The blood-brain barrier is regulated at the level of cerebral vasculature, pericytes, and astrocytic endfeet sealed by tight junctions and low rates of transcytosis. The choroid plexus maintains the blood-CSF barrier, bridging systemic circulation to the CSF, through tight junctions at the apical aspect of epithelial cells, transcytosis, active transport, metabolism[135], and regulation of endothelial fenestration[62]. Both of these brain barriers change across time-of-day, although even less is known about the circadian responses in these barrier functions and their cellular components (Fig. 2). Therefore, considering daily change in these barriers will be key for access and control over brain borders, including actively bypassing them, as a goal of pharmacology[171,172].

Inherent variation across the day in the blood-CSF barrier have been identified at the level of tight junctions, drug transport, and metabolism. In mice, tight junctions at the apical aspect of choroid plexus epithelial cells widen during the rest phase and pull epithelial membranes tighter together during the active phase, which correlated with changes in gene expression of barrier-associated genes like integrins, cadherins, and sorting nexins[135]. Further, drug transporters may also be differentially expressed across the day like ABCC4 (MTX transporter)[168] and ABCG2 (Donepezil transporter)[167]. Other diurnally regulated transporters may interact with pathogens or toxins like ABCF3 (flavivirus antiviral transporter) and SLC7A8 (thyroid hormone and methylmercury transmembrane transporter)[135]. The daily change in choroid plexus metabolic processes, including an increase in oxidative metabolism during the active phase[135], can interface with both ATP-dependent active transport across the barrier and drug pharmacology. More broadly, metabolism and mitochondrial function are disrupted in the brains of individuals at high risk for psychosis[173,174] and altered bioenergetics profiles are hallmarks of late-onset Alzheimer's disease[175], suggesting broad overlap in daily metabolism change and long-term brain health. Aβ uptake capacity of the choroid plexus may also depend on appropriate daily cycling as rhythmicity is observed in genes or gene products that interface with amyloid-β (Aβ), including ACE and TTR[135,176],

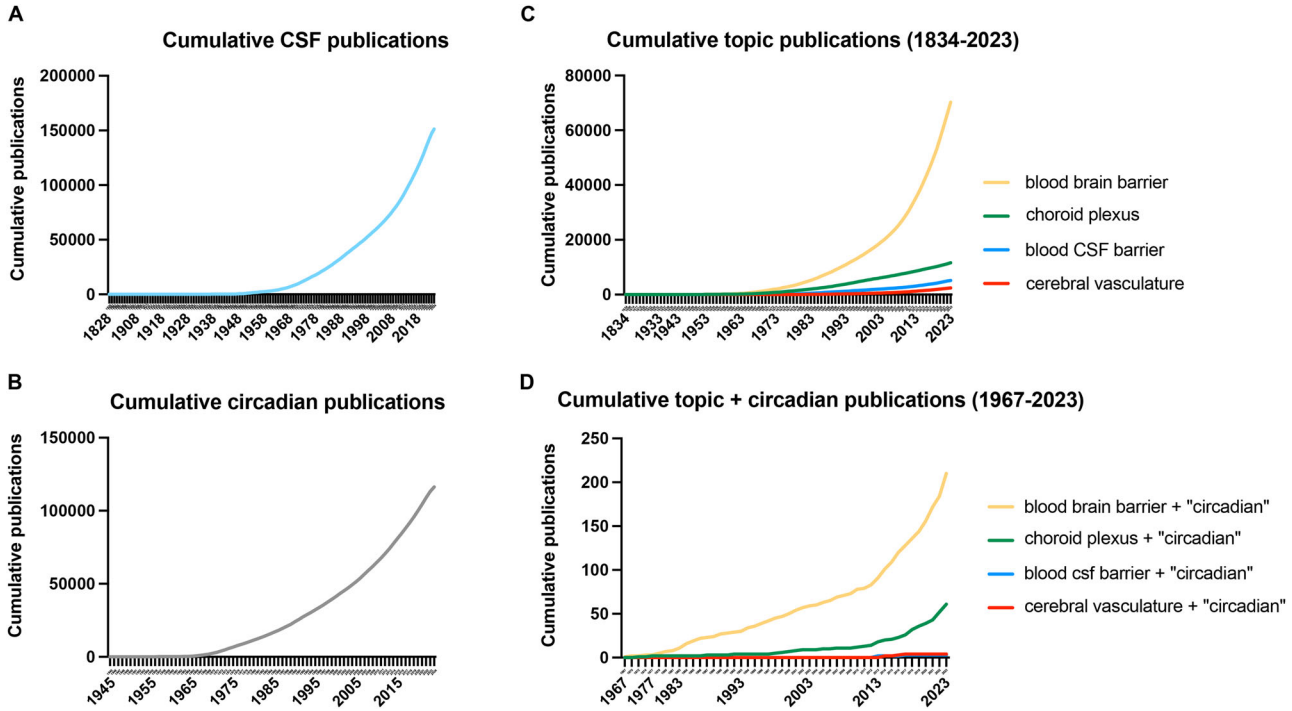

Fig. 2 | Circadian aspects of the cerebrospinal fluid (CSF) system are relatively understudied. Despite large increases in studies about CSF (**A**) and circadian systems (**B**), only the blood-brain barrier is deeply studied at the intersection of systemic circulation, the brain, and circadian rhythms (**C**, **D**). This emphasis on blood-brain barrier overshadows choroid plexus, blood-CSF barrier, and cellular components of the blood-brain barrier like cerebral vasculature. Thus, representing a scientific opportunity to increase the study of the CSF system across diurnal changes. Search terms on PubMed of "cerebrospinal fluid," "circadian," "blood brain barrier," "choroid plexus," blood CSF barrier," "cerebral vasculature," "blood brain barrier + circadian," "choroid plexus+ circadian," blood CSF barrier+ circadian," "cerebral vasculature+ circadian." Data exported 4 September 2024 and plotted until 2023.

and apolipoprotein J and gelsolin[177]. Increased CSF Aβ during sleep deprivation in healthy middle-aged adults has been shown to be dependent on sleep disruption and not due to stress or a more general circadian disruption[178]. Cycling Aβ uptake was observed in a human choroid plexus papilloma cell line that was incubated with Aβ-488[176] and FACS purified across 24 h, although direct measures of choroid plexus Aβ uptake across the day remain to be measured in vivo. Further, in the Alzheimer's model (APP/PS1), choroid plexus *Per2* rhythmicity has been shown to change in some rodents (year-old males), but not in others (females, young males) or even in other core circadian gene expression like *Cry2*[165], so the effects of Alzheimer's disease and CSF Aβ on choroid plexus rhythmic gene expression and function remain an active field of study.

Pharmaceutical interventions can also directly change the circadian rhythms of the choroid plexus and brain ventricular system. As noted earlier, dexamethasone can resynchronize the choroid plexus molecular clock[135,160,161]. Anesthetics interface with diurnal behaviors, including arousal and diurnal processes in the brain like the CSF exchange with brain parenchyma or clearance of brain tracers to the periphery that are implicated in glymphatic function[58,179,180]. Sevoflurane is an inhaled anesthetic that induces hypnosis, amnesia, analgesia, akinesia, and autonomic blockade. When sevoflurane is administered to animals, changes in choroid plexus *Per2* rhythmicity, but not SCN *Per2* rhythmicity, were observed[181] by explant readouts from *Per2*-Luciferase mice. Intriguingly, this shift was detectable up to 30 days after treatment, indicating long-term modulation of choroid plexus rhythmicity[181]. When LiCl was applied to choroid plexus explants, it delayed the in vitro circadian clock phase and prolonged the period, circadian disruptions that were opposite from those observed after CHIR-99021 (a glycogen synthase kinase-3 inhibitor) application[182]. The LiCl-induced phase delay and period lengthening were lessened by the additional application of chelerythrine (a protein kinase C (PKC) inhibitor), suggesting a PKC-involved mechanism[182]. Thus, attention to time of day is recommended when administering circadian-disrupting drugs at the brain CSF barrier, like dexamethasone, LiCl, or anesthetics.

Conclusions and open questions. From the experiments inducing behavioral changes by transplanting CSF between sleep-deprived or food-restricted animals over 100 years ago, the CSF system is emerging anew as a critical component of daily brain health and function. Our ability to understand daily changes in this system is growing as modern tools emerge like CSF fiber photometry[135] and intravital choroid plexus imaging[183] enable real-time monitoring CSF and choroid plexus in animal models coupled with the availability of large-scale sequencing. The daily changes summarized here that have been observed in CSF composition, CSF volume/pressure, choroid plexus activity, and brain barrier permeability and metabolism solidify the CSF system as a key circadian element.

The field remains very active and key open questions remain as to both upstream influences on the rhythmicity and downstream functions of rhythmic components of the CSF system. For example, any observation of diurnal changes does not always implicate true circadian rhythmicity—thus, functional dependence on light cues and the molecular clock should be validated for diurnal observations. This question of true circadian rhythmicity and entrainment stimuli is important for intervention, as the answer to it provides details on how best to manipulate the system in disease or to harness CSF for drug delivery. Some signals, like CSF TTR have been shown to continue to cycle in total darkness for multiple days and in ex vivo explants[135], however this isn't the case for all diurnal components of the CSF system and activity, feeding, hormones, and sleep can all interact with and tune the system. Some studies have begun to separate these components by removing *Bmal1*[135,152], lesioning the SCN[160], modulating hormones[153,159], inverting feeding times[135], measuring changes that continue in the dark[135,152], and comparing CSF pressure between diurnal humans and crepuscular

rodents[142]. The challenge of metabolism, in particular, is complex as it can directly interact with mechanisms of active transport due to its influence on ATP availability and direct interface with translation[184]. New studies should continue to work to separate sleep, the cellular clock, feeding, activity, and light cues when reporting daily changes in CSF and choroid plexus properties so that interventions that take advantage of the blood-CSF barrier or CSF production/clearance can be appropriately timed to extrinsic cues. Modulation of diet, light exposure, or activity could enhance pharmacologic interventions, as has been shown relevant for immunology, cancer progression, and cognition (e.g., refs. 2,185).

Further, additional functions ascribed to the CSF system, including the CSF solutes that support the developing and adult neural stem cell niches, are likely to be affected by circadian rhythmicity. Age-dependent differences in circadian regulation of the CSF system, from the inception of CSF rhythmicity to natural aging, likely respond to the developing and aging body clock and may contribute to changes in neurological function across the lifespan. The reduced CSF production associated with aging[126] could, for example, result from overall lessened circadian regulation with age. The crossover of brain development and aging, including brain stem cells, with circadian regulation could inform regenerative strategies and complement the understanding of the cognitive effects of circadian disruption.

Continued collaboration among circadian experts, those in the brain borders fields, and clinicians is critical for the rigorous study of circadian changes in the interfaces between the brain and systemic rhythmicity. Large-scale human studies of CSF imaging via MRI, CSF component analysis (like harnessing time stamps from emergency department visits with banked CSF from lumbar puncture), and analysis of postmortem choroid plexus or CSF samples with time-of-day information will further enable more translational conclusions from controlled studies in models, including identifying common principles among model organisms with diverse photic periods[28–30]. The most critical functions of the choroid plexus and CSF system, including CSF production and turnover, solute control like release of factors that support the brain or clearance of waste products, and maintenance of the blood-CSF barrier are crucial to understand from the perspective of circadian rhythms.

Ultimately, considering the circadian nature of the choroid plexus and CSF system complements current efforts to harness this system for therapeutic intervention. For example, harnessing natural daily variation in the blood-CSF barrier could aid efforts to time approaches that bypass the blood-CSF or blood-brain barrier for drug delivery[186–188]. Further, approaches that target gene therapy to the blood-CSF barrier, e.g., AAV transduction of ependymal or choroid epithelial cells, which has been shown to improve neurologic symptoms in rodent models of Huntington's disease, lysosomal storage disorders, and Alzheimer's disease[189–192], could restore any circadian functions that are ultimately determined to be key for behavioral readouts. In addition to restoring healthy circadian function, the ability of the choroid plexus to secrete proteins into the CSF can be harnessed to restore CSF solute composition regardless of the endogenous source or to add metabolic function to neutralize toxins or locally convert an inactive prodrug to an active drug. While many correlates of CSF and choroid plexus with circadian rhythm have been determined as described here, the next frontier is functional evaluation of which of these molecular or radiological biomarkers are reporting circadian state vs. those that are controlling circadian output or brain phenotypes. Careful functional evaluation will then open the possibility of harnessing the choroid plexus and CSF system to both monitor circadian changes and intervene to restore or supplement function.

Data availability

No datasets were generated or analyzed during the current study.

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Competing interests

The author declares no competing interests.

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