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REVIEW

The cerebrospinal fluid: regulator of neurogenesis, behavior, and beyond

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Abstract The cerebrospinal fluid (CSF) has attracted renewed interest as an active signaling milieu that regulates brain development, homeostasis, and disease. Advances in proteomics research have enabled an improved characterization of the CSF from development through adulthood, and key neurogenic signaling pathways that are transmitted via the CSF are now being elucidated. Due to its immediate contact with neural stem cells in the developing and adult brain, the CSF's ability to swiftly distribute signals across vast distances in the central nervous system is opening avenues to novel and exciting therapeutic approaches. In this review, we will discuss the development of the choroid plexus-CSF system, and review the current literature on how the CSF actively regulates mammalian brain development, behavior, and responses to traumatic brain injury.

Keywords Cerebrospinal fluid · Choroid plexus · Neurogenesis · Traumatic brain injury

Abbreviations

BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
BMP	Bone morphogenetic protein
Bmpr1a	BMP receptor 1a
BrdU	Bromodeoxyuridine
CNS	Central nervous system
ChP	Choroid plexus
CSF	Cerebrospinal fluid
DG	Dentate gyrus
ELISA	Enzyme-linked immunosorbent assay
ERK1/2	Extracellular signal-regulated kinase 1/2
Ecrg4	Esophageal cancer-related gene-4
FGF	Fibroblast growth factors
GDF	Growth differentiation factors
GDNF	Glial-derived neurotrophic factor
IGF	Insulin-like growth factors
IGF1R	IGF1 receptor
IGFBPs	IGF-binding proteins
Irs2	Insulin receptor substrate 2
LIF	Leukemia inhibiting factor
Lmx1a	LIM homeobox transcription factor 1, alpha
LV	Lateral ventricle
MS	Mass spectrometry
NEP	Neuroepithelium
NGF	Nerve growth factor
NT-3	Neurotrophin 3
Pdk1	Phosphoinositide-dependent kinase-1
PEDF	Pigment epithelial derived factor
Pten	Phosphatase and tensin homolog
RA	Retinoic acid
Rhomb	Rhombencephalon
sAPP	Soluble amyloid precursor protein
Shh	Sonic hedgehog
SCN	Suprachiasmatic nucleus
SVZ	Subventricular zone
Tel	Telencephalon
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TBI	Traumatic brain injury

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V	Ventricle
VEGF	Vascular endothelial growth factor
VZ	Ventricular zone
Wnt1	Wingless-type MMTV integration site family, member 1

Introduction

The cerebrospinal fluid (CSF) has intrigued scientists, physicians, and philosophers for close to 4,000 years. The earliest documentation of a fluid within the head dates back to the ancient Egyptians, dating back to 1700 B.C. [1]. It was the early Greeks, however, who took on the first known investigations of the CSF. Hippocrates (460–370 B.C.) described 'hydrocephalus,' a condition that he thought was caused by too much 'water in the head' [1, 2]. Subsequently, circa 300–250 B.C., Herophilus is credited for being the first to describe the choroid plexus and named it "chorioid mennix" due to the vascular similarity to the "chorioid" of the fetus [3]. Herophilus and Erasistratus, both anatomists interested in the human body, are credited for being the first to describe the ventricles of the brain and suggested they play a role in muscular contraction [2]. However, it is believed that Galen of Pergamon (circa A.D. 150), due to his fame and popularity, may have had the most historical influence on describing the function of the ventricles and the CSF by studying Ox brains [1, 2]. He described an alchemical transformation that happened at the base of the brain as a 'vital spirit' coming from the blood vessels that was transformed into the 'animal spirit' and emerged into the ventricles. This 'animal spirit' was then carried through the nerves to contract the muscles of the body and energize the entire physical being [2]. Galen's prevailing theory of a fluid within the ventricles as a spirit (pneuma) lasted for over 1,500 years, until the rebirth of human anatomy at the time of the Renaissance [2]. Today, we know that the CSF is produced primarily by the choroid plexus, located in each ventricle in the brain. Elucidating the full range of CSF functions continues to be an area of research under intense investigation today [4–6].

The choroid plexus, the intraventricular organ

The choroid plexus is a highly vascularized secretory epithelium that begins to form shortly following anterior neural tube closure. It arises from an invagination of the dorsal roof plate along the midline of the neural tube and develops in vertebrate ventricles in a sequential manner [7]. It first can be visualized as epithelial invaginations that emanate from the single-layered roof plate of the hindbrain that create the fourth ventricle choroid plexus at approximately embryonic mouse age E11–E12 [7]. Subsequently, midline invaginations from the area chorioidea, an area of the medial wall named by His, then forms the anterior and posterior domains of the telencephalic choroid plexus, which extend into the lateral ventricles (E11–E12) [7]. Finally, the diencephalic choroid plexus forms in the third ventricle at approximately E12–E14 in the mouse [3, 8]. Subsequently, the continuity between the telencephalic and diencephalic choroid plexuses develops [9]. During mouse embryogenesis, the choroid plexus is clearly visible as an observable outgrowth already at E12.5 in the hindbrain ventricle. In the human embryo, the choroid plexus begins to develop at approximately 44 days post-ovulation in the fourth and lateral ventricles and is quite large already at 9 weeks gestation, filling a large part of the lateral ventricle and spanning the length of the fourth ventricle (Fig. 1) [10]. One of the earliest molecular markers of choroid plexus differentiation is the expression of Transthyretin [11].

The choroid plexus consists of highly fenestrated capillaries, which are essentially microvessels with portions of the capillary wall sealed by thin diaphragms, mesenchymal cells, and epithelial cells. The secretory epithelial cell layer is derived from neuroepithelial cells lining the ventricles during development. The epithelial cells contain tight junctions that already function very early in development and prevent the passive diffusion of molecules into the CSF from the choroidal vasculature and interstitial space [12, 13]. As such, the choroid plexus actively secretes CSF into the ventricles and creates the blood–CSF barrier.

The localized formation of the choroid plexus is regulated by a number of growth and transcription factors. In the hindbrain, the choroid plexus develops from the lower rhombic lip that is specified by high levels of bone morphogenetic protein (BMP) signaling, and demarcated by the expression of growth factors Wnt1, and Gdf7, and the transcription factor Lmx1a [14–20]. Loss-of-function and gain-of-function studies have also shown that Sonic hedgehog (Shh) originating from fourth ventricle choroid plexus epithelial cells promotes proliferation of fourth-ventricle choroid plexus progenitor cells [18] as well as the choroid plexus mesenchyme [19]. For lateral ventricle choroid plexus development, the BMPs specify choroid plexus cell fate within the dorsal midline, as demonstrated by loss of choroid plexus in mice with conditional deletion of the BMP receptor 1a (Bmpr1a) within the developing telencephalon using the Foxg1-Cre allele, a gene expressed throughout the telencephalon and along the dorsal midline [21]. In addition, the BMPs generate an activity gradient at the dorsal midline telencephalon that induces telencephalic choroid plexus differentiation [22]. The anterior and posterior domains of the telencephalic choroid plexus can be further distinguished by Gdf-7, whose expression localizes to the anterior domain [16]. During normal chick

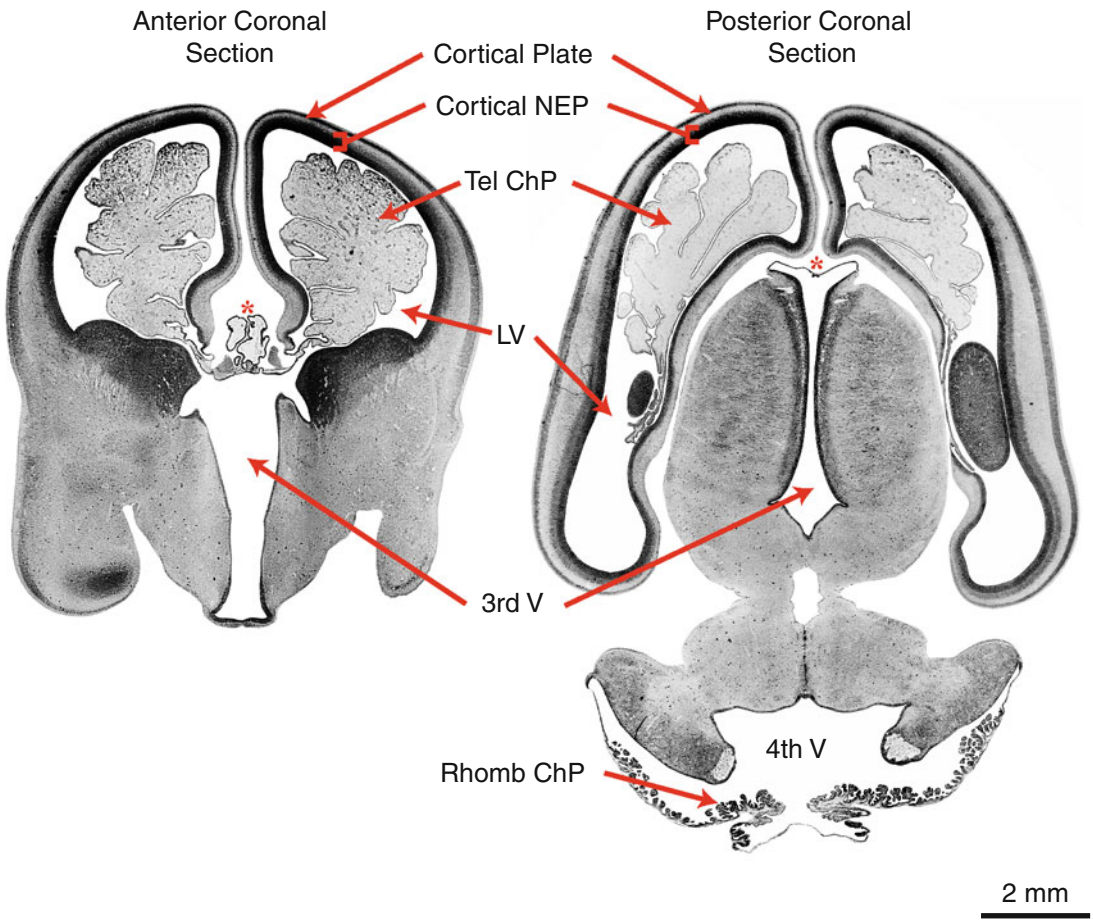

Fig. 1 Coronal section of the human embryonic brain at gestational week 9. *Left panel* At gestational week 9 in the human embryo, the telencephalic choroid plexus occupies a large portion of the lateral ventricle. The choroid-plexus-secreted CSF in the lateral ventricles bathes the cortical neuroepithelium, thereby regulating neurogenesis and the formation of the cortical plate. *Right panel* a more posterior view of the developing human embryo at gestational week 9 shows that the lateral ventricle continues to be filled by the choroid plexus. The fourth ventricle choroid plexus is observed to extend along the length of the fourth ventricle. *Asterisks* denote third ventricle choroid plexus. *ChP* choroid plexus; *LV* lateral ventricle; *NEP* neuroepithelium; *Tel* telencephalon; *Rhomb* rhombencephalon; *V* ventricle. Reprinted with permission from the authors, Bayer SA, Altman J [10]

development, the telencephalic choroid plexus can be distinguished from surrounding neuroepithelial tissue as early as embryonic day 4 (E4), when the developing choroid plexus expresses Bmp7 and Otx2, lacks expression of both Emx1 and Emx2 in neighboring cells, and can be clearly differentiated by E6 with the expression of Transthyretin [11].

The cerebrospinal fluid and its functions

Although the study of the CSF dates back to the early Egyptians and Greeks, it was not until about 100 years ago that Harvey Cushing observed that the choroid plexus secretes CSF into the brain's ventricles [23], casting aside lingering views of the CSF as a possible post-mortem precipitate. It is estimated that an adult human circulates 150 ml of CSF throughout the central nervous system (CNS), and that the CSF turns over about three to four times per day, suggesting that an adult human produces approximately 500 ml of CSF daily. In the adult brain, the CSF flows from the lateral ventricles to the third ventricle, fourth ventricle, and then to the spinal cord and subarachnoid space (Fig. 2). Its flow is propelled by three main forces: (1) arterial pulsations in the choroid plexus, (2) a hydrostatic pressure gradient, and (3) ciliary motion of the ependymal cells lining the ventricles [12]. While the CSF is 99% water, provided primarily by Aquaporin-1 channels located on the apical membrane of the epithelial cells, the CSF is also a rich source of proteins, ions, lipids, hormones, cholesterol, glucose, and many other molecules and metabolites. Early work on the CSF focused primarily on the importance of pressure homeostasis in the developing brain [24–26]—too little pressure decreases brain size [24], while too much pressure leads to impaired neural stem cell division and hydrocephalus [27–29].

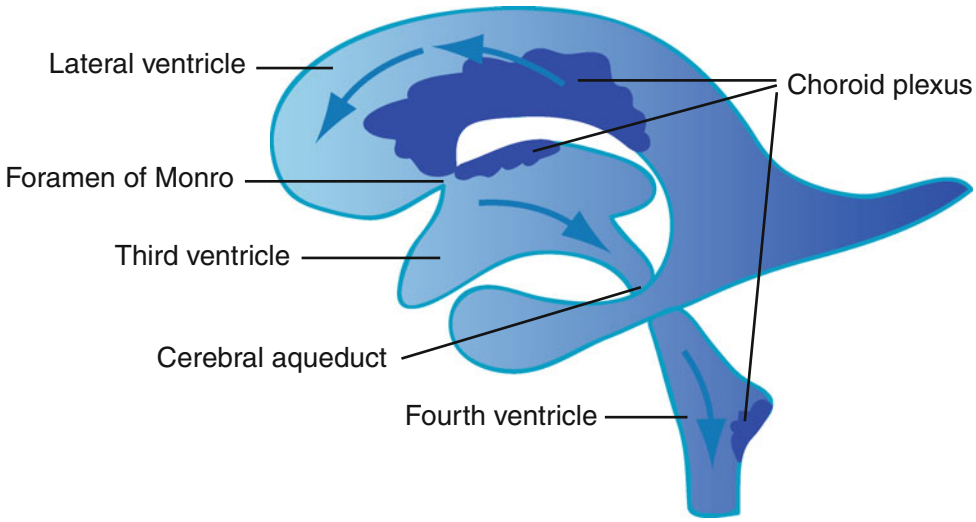

Fig. 2 Sagittal schematic of CSF flow in the mature cerebroventricular system. CSF is produced primarily by the choroid plexus, which is located in each ventricle in the brain. CSF flows from the lateral ventricles, via the foramen of Monro, to the third ventricle, and then through the cerebral aqueduct/aqueduct of Sylvius to the fourth ventricle. Upon leaving the fourth ventricle, the CSF flows down the spinal cord and fills the subarachnoid space surrounding the brain. It is ultimately resorbed into the venous circulation by arachnoid villi

More recent work has turned to investigating the biological functions of known CSF-resident factors. Many of these CSF-distributed factors have been speculated to influence a wide range of behaviors including sleep and appetite. For example, early experiments showed that CSF collected from sleep-deprived goats induces states of deep sleep for 12–24 h when infused into the cisterna magna of control laboratory cats [30]. In an attempt to isolate the molecule, Lerner and colleagues purified a brain fatty acid from the CSF of sleep-deprived cats [31], which was later identified as oleamide (*cis*-9,10-octadecenoamide) [32]. Further studies revealed that oleamide levels are elevated in the CSF of sleep-deprived rats [33]. When synthetic oleamide is injected into laboratory rats either intraventricularly or intraperitoneally, it decreases sleep latency and induces physiologic sleep [33, 34]. The exact mechanism by which oleamide induces sleep remains to be elucidated. However, oleamide binds to cannabinoid (CB1), GABA, and serotonin receptors [35–37], and is thought to modulate its hypnotic function through these interactions.

An arousal-promoting factor has also been found in the CSF. Orexin-A (also known as hypocretin-1), a protein released by lateral hypothalamic neurons and a key regulator of wakefulness [38–40], is present at lower levels in the CSF of narcoleptic patients than unaffected individuals [41]. Orexin infusion into the lateral ventricles elicits prolonged wakefulness in rats that are awake [42–44] and stimulates arousal from sleep [45]. Interestingly, orexin infusion into different ventricles appears to evoke distinct behavioral responses. For example, in contrast to the pronounced waking response stimulated by lateral ventricle infusions, orexin infusion into the fourth ventricle leads to weaker effects on arousal [45], suggesting that the sites of CSF-orexin action flank, or lie close to the lateral and/or third ventricular walls, consistent with known targets of orexin projections.

In addition to sleep- and arousal-inducing factors in the CSF, several CSF-distributed factors play important roles in circadian rhythms and associated locomotor activity. For example, encapsulated suprachiasmatic nucleus (SCN) transplants to the third ventricle wall can restore circadian rhythms in hamsters whose own SCN has been ablated [46]. Although the specific SCN-secreted factors were not identified in the above-mentioned study, additional clues have since emerged, including the identification of TGF-α [47] and cardiotrophin-like cytokine [48]. When infused into the third ventricle, both of these factors disrupt circadian locomotor activity. TGF-α infusion also impairs circadian rhythmicity, an effect mediated by the EGF receptors expressed by hypothalamic subparaventricular zone neurons [47]. In addition, melatonin, a hormone that regulates circadian rhythms, is released by the pineal gland into the CSF [49, 50]. Melatonin is a potent free radical scavenger and antioxidant, and it is believed that one of the major roles of melatonin in the CNS is to behave as a neuroprotective agent that decreases oxidative stress and removes reactive oxygen species from the CNS [51]. Interestingly, patients with severe traumatic brain injury have been reported to have increased CSF melatonin levels that correlate with markers of oxidative stress [52], suggesting a possible homeostatic role for melatonin in the CSF.

CSF-borne factors have also been shown to affect appetite. When CSF collected from the lateral ventricles of fasted "donor" sheep is infused into the ventricles of satiated "recipient" sheep, increased feeding is observed. The converse experiment holds true as well, such that when satiated "recipients" receive CSF from satiated "donors," the recipients feed less [53]. Although the exact CSF-distributed factors mediating feeding remain to be elucidated, several candidates have been suggested. Insulin injection into the lateral ventricles, for example, elicits decreased food intake in baboons [54]. In addition, low

CSF-leptin:serum-leptin ratios have been reported to correlate inversely with body mass index (BMI) in some individuals [55], raising the possibility that impaired leptin transport across the blood–brain barrier could underlie increased BMI [55]. Therefore, due to its intimate contact with the thalamus, hypothalamus, and pineal gland, the CSF may convey important peptide signals regulating behavioral states and other states of consciousness as well.

Spatial gradients of CSF proteins have also been shown to guide the behavior of distinct cell populations, including the migration of neuroblasts from the subventricular zone (SVZ) to the olfactory bulb in the adult brain [56]. In these studies, the ciliary beating of ependymal cells was shown to be crucial for providing appropriate Slit chemorepellent activity for the migrating neuroblasts [56]. Taken together, CSF-distributed factors play an active role in many aspects of normal adult brain activity, setting the stage for investigating how the CSF may dynamically regulate development as well as disease.

Brain development and the cerebrospinal fluid

Recent studies have taken advantage of advances in proteomic tools including mass spectrometry to better characterize the CSF proteome. These studies have demonstrated that the CSF proteome consists of hundreds of proteins of the extracellular matrix, regulators of osmotic pressure, ion carriers, hormone-binding proteins, regulators of lipid metabolism, and various enzymes and their regulators [57–61]. The CSF shares many similarities across species [57–61], facilitating a multi-tiered experimental approach across many laboratories for the study of CSF including zebrafish, chick, rodents, marsupials, and others, on brain development. While the classical model of diffusion through tissues certainly holds true [62, 63], the apical, ventricular enrichment of phosphotyrosine [64] and phospho-ERK1/2 [65] activities in cortical progenitor cells have raised the provocative idea that growth factor signaling originates from the CSF as well. Indeed, a flurry of recent studies demonstrates the dynamism of embryonic CSF during brain development. CSF alone can promote the development and growth of neural stem cells and cortical explants [66]. Although the factors responsible for the favorable effects of embryonic CSF are only beginning to be uncovered, the range of factors already known to be active in CSF during embryonic brain development includes fibroblast growth factors (FGFs) [67], insulin-like growth factors (IGFs) [66, 68], sonic hedgehog (Shh) [69], retinoic acid (RA) [66, 70], bone morphogenic proteins (BMPs) [66], Wnts [66], and others [71, 72] shown in Table 1.

FGF2, IGF2, and Shh in CSF have been demonstrated to stimulate proliferation of progenitor cells in the brain, with each factor having a distinct known cellular target to date.

Table 1 Growth factors present in embryonic CSF

Growth factor	Method of detection	Species	References
Amyloid beta A4 protein precursor (APP)	Mass spectrometry (MS)	Rat, Human	[60]
BMPs	Luciferase assay	Rat	[66]
FGF2	Western blot	Chick	[67]
GDF-3	MS	Rat	[66]
GDF-8	MS	Rat	[66]
IGF-1	ELISA/western blot	Mouse, Rat	[66, 68]
IGF-2	MS/western blot	Mouse, Rat	[66]
Leukemia inhibitory factor (LIF)	ELISA	Mouse	[72]
NGF	ELISA/western blot	Chick	[71]
Pigment epithelial derived factor (PEDF)	MS	Human	[60]
Retinoic acid	HPLC-MS, cell based assay	Chick, Rat	[66, 70]
Shh	ELISA	Mouse	[69]
Wnts	Cell based assay	Rat	[66]

For example, immunodepletion of embryonic chick CSF-FGF2 reduces progenitor proliferation of chick midbrain progenitors [67, 73, 74]. Intravascularly injected FITC-conjugated FGF2 passes into the embryonic CSF [67], suggesting that somatic sources with access to CSF may regulate neurogenesis as well. The following sections will summarize findings for IGFs, Shh, RA, as well as other factors.

Insulin-like growth factor 1 and 2 signaling in the cerebrospinal fluid

The IGFs (IGF1 and IGF2) are a class of growth factors present in the embryonic CSF [66, 68, 75, 76]. Well established in the regulation of prenatal growth and body size [77–79], the IGFs bind with strongest affinity to the IGF1 receptor (IGF1R) to stimulate proliferation [80]. Conditional deletion of IGF1R in neural precursors leads to microcephaly [66, 81, 82]. In contrast, IGF1 overexpression promotes S-phase commitment, accelerated cell cycle kinetics, and cell survival, leading to hyperplasia [82–85]. Depending on cellular context, IGF1 can also regulate neuronal differentiation, glial development, and cell size [86]. Downstream of IGF1R signaling, several mouse mutants can be leveraged for the study of brain size, including Irs2 [87], Pdk1 [88], and Pten [89].

IGF2 endows the CSF with robust growth and survival-promoting effects during neurogenesis [66]. A transient spike in CSF-IGF2 levels at middle-to late-stages of neurogenesis (E16–19 in rat; E16–18 in mice) stimulates the proliferation of neural precursor cells in explant cultures of the developing cortex [66], as well as the growth and maintenance of neurospheres, an in vitro model of neural stem cells [90, 91]. CSF-IGF2 binds to the apical membrane and primary cilia of cortical progenitors, and the proliferation-inducing effects of CSF appear IGF2-dependent, as gain-of-function and loss-of-function experiments in vitro in explants and neurospheres produce opposing effects on proliferation. Igf2-deficient mice have a defect in neurogenesis affecting the uppermost layers of the cortex [66]. The choroid plexus expresses IGF2 [92, 93], though other sources of IGF ligands exist, including the developing vasculature [94], neighboring cells, and perhaps even somatic sources. Studies involving intracerebroventricular injections of IGF1, IGF1-neutralizing antibodies, and IGF1R inhibitors further demonstrate that IGF signals delivered by the embryonic CSF trigger proliferative events in the cortical ventricular zone (VZ) [84].

The effects of CSF-IGF2 are also age-dependent [66]. CSF-IGF2 levels in rodents are modest at the earliest stages of neurogenesis, peak near the end of neurogenesis, and decrease postnatally, suggesting that IGF2 may regulate specific aspects of neurogenesis. Intriguingly, in *Drosophila*, insulin/IGF-like peptides secreted by glia stimulate quiescent neuroblasts to reenter the cell cycle in *Drosophila* [95, 96]. An analogous role of IGF signaling in the mammalian brain is an interesting idea that has not yet been explored.

Sonic hedgehog signaling in the cerebrospinal fluid

Sonic hedgehog (Shh), a morphogen well established in ventral brain and cerebellar development, is also found in the CSF. The hindbrain choroid plexus epithelial cells secrete Shh into the CSF [18, 69], and this CSF-Shh has been suggested to stimulate the expansion of a progenitor domain in the hindbrain choroid plexus [18]. Interestingly, Shh also signals directly to choroid plexus pericytes to direct vascular growth, an essential component of normal choroid plexus development and expansion [19]. Pericyte signals may subsequently influence both progenitor proliferation as well as angiogenesis [19]. Thus, more detailed mouse genetic approaches will elucidate the exact impact of Shh on distinct cell lineages and on choroid plexus development. In addition to this tissue-autonomous role for choroid-plexus-secreted-Shh, CSF-Shh appears to be a key mitogen for proliferating cerebellar granule precursors. In this context, Shh was previously thought to be produced primarily by Purkinje cells [97–99]. However, Wnt1-Cre-mediated deletion of Shh in the hindbrain choroid plexus also impairs proliferation of cerebellar granule neuron precursors [69]. These findings support a model in which choroid plexus borne Shh may also act in a paracrine manner to instruct cerebellar development.

Retinoic acid in the cerebrospinal fluid

Retinoic acid (RA), a hormone signal derived from vitamin A, has been identified in CSF [6, 66, 70]. RA provides key long-range signaling activity for the developing brain [100–102]. RA synthetic and catabolic enzymes are expressed in the choroid plexus [66] as well as the meninges [103]. Thus, the meninges are an important source of RA as well. Foxc1 mutant mice, which have reduced meningeal RA secretion, have diminished expansion of the neuroepithelium and decreased numbers of intermediate progenitor cells [103]. While choroid-plexus-derived-RA is secreted directly into ventricular CSF, meningeal sources of RA may reach the neuroepithelial cells via the lateral ventricular CSF as well as by crossing the cerebral mantle. The roles of meningeal-RA suggest a wide range of mechanisms by which signals at both the basal process [104, 105] and apical process may play key roles in the maintenance of the apical progenitors [5].

Other potential signaling activities in the cerebrospinal fluid

The CSF is home to hundreds of proteins and signaling activities, providing an elaborate range of biological functions for this complex fluid. For example, Wnt signaling, which plays a fundamental role in regulating early development of the CNS [106–108], has been identified in embryonic CSF [66]. Similarly, dynamic BMP activity [21, 109] is also found in the CSF [66]. Consistent with the coordination of BMP signaling by the choroid plexus-CSF system, growth and differentiation factors 3 and 8 (Gdf3 and Gdf8), both members of the TGF-β superfamily of proteins that can modulate BMP signaling [110], are found in the CSF [66]. Our knowledge of CSF-distributed signals likely represents the tip of the iceberg with regard to active signaling in this highly dynamic fluid (Fig. 3).

The cerebrospinal fluid and brain injury

The CSF has also attracted interest in the field of brain injury. The CSF utilizes volume transmission, or bulk flow, of neurogenic and neuroprotective factors believed to have

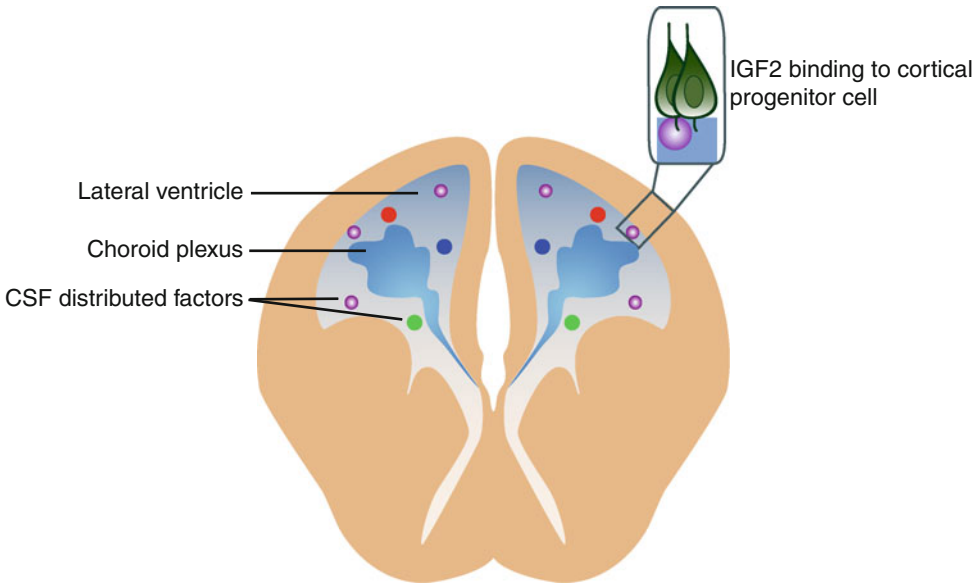

Fig. 3 Coronal schematic of choroid plexus-secreted factors distributed in the CSF during embryonic cortical development. IGF2 (represented by *purple spheres*) and other factors secreted by the choroid plexus are delivered to target cells on the apical, ventricular surface of the developing cerebral cortex

therapeutic effects following brain injury. The continuous exchange of CSF also serves as a "sink" that flushes the ventricular system of debris and injury products. The choroid-plexus-CSF system can be disrupted in a wide range of CNS injuries, from hydrocephalus to age-associated neurologic diseases (e.g., Alzheimer's disease) [12, 111]. For the purposes of this review, we will focus primarily on brain injury via traumatic or ischemic events.

There are a number of mechanisms that may occur during brain injury that disrupt the choroid plexus-CSF (CP-CSF) homeostasis [111]. Some of the experimental models for investigating traumatic brain injury (TBI) typically include: lateral head movement acceleration, exposure to a blast, penetrating brain injury, and impact acceleration. These injury approaches induce damage to the choroid plexus, alterations in CSF secretion, increased leukocytes within the brain and CSF, decreased clearance of toxins, catabolites and proteins, and therefore diminished supply of nutrients and growth factors to the brain [111]. It is now believed that recovery from brain injury involves the gradual repair of these mechanisms in order to return the CP-CSF system to homeostasis to promote neuroregeneration and neuroprotection [111].

Growth factors in CNS injury

A number of growth factors are upregulated in the brain and CSF following injury. These include, but are not limited to, IGF1 [112–114], IGF2 [115], FGF [116, 117], nerve growth factor (NGF) [118, 119], transforming growth factor-beta (TGF-β) [120–122], glial-derived neurotrophic factor (GDNF) [123], brain-derived neurotrophic factor (BDNF) [112, 124, 125], Neurotrophin 3 (NT-3) [112, 126, 127], Neurotrophin 4/5 [128], vascular endothelial growth factor (VEGF) [129], erythropoietin [130–132], tumor necrosis factor-alpha (TNF-alpha) [133–135], and soluble amyloid precursor protein (sAPP) [136, 137] (Fig. 4; Table 2). Recently, NGF was found to be elevated in the CSF of children post TBI at both 2 and 48 h post injury. In addition, increased NGF levels, together with lower IL-1β levels, were reported to correlate with improved neurological outcomes [118, 138, 139].

The IGF signaling pathway plays an important role in models of brain injury. IGF1 was shown to be upregulated and to localize to the site of cortical contusion up to 48 h post-injury in a mouse model of cortical impact brain injury [113], suggesting a possible role in neuroprotection and neurogenesis by IGF1. Previous cortical contusion studies in rats also showed similar results with Igf1 mRNA increasing at the site of injury and peaking by day 3 [114]. Although these studies did not assess changes in IGF1 within the CSF, they indicate that the IGF signaling pathway may be an important modulator in TBI models. This observation is further supported by studies with IGF2. IGF2 is highly expressed in the choroid plexus, meninges, and CSF throughout development and into adulthood [140–142]. As mentioned above, increased IGF2 levels in vitro increase proliferation of neural stem cells both in cortical explant cultures and isolated neural stem cells [66]. In addition, the choroid plexus is also known to secrete IGF binding proteins (IGFBPs), which assist in the transportation of IGFs and also are believed to modulate their activity [143]. In adulthood, it is thought that IGF2 levels in the CNS remain relatively stable [140, 141]. However, evidence suggests that IGF2 may be an important regulatory molecule released in a penetrating model of TBI [115]. Walter and colleagues exposed adult rats to a cortical

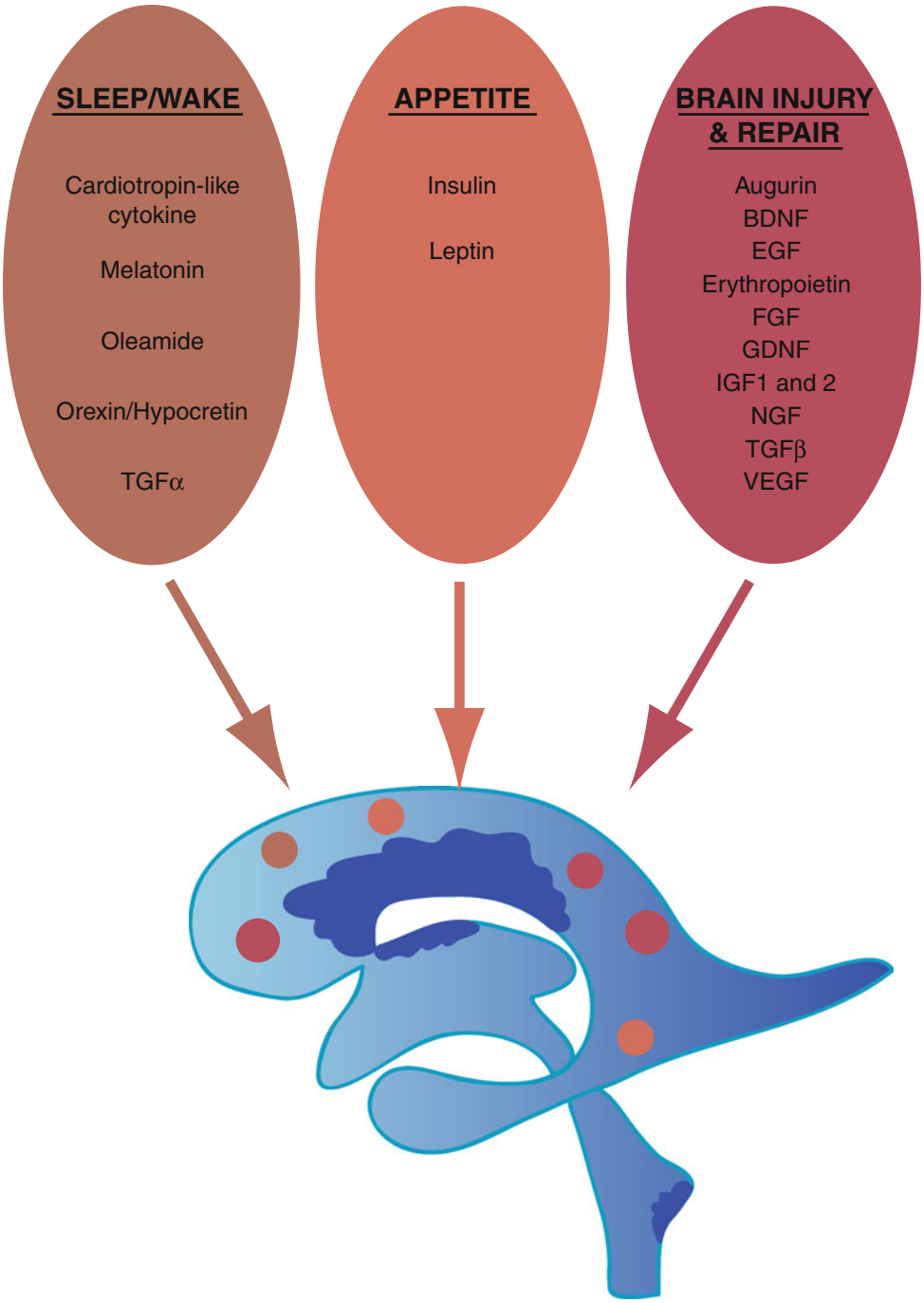

Fig. 4 Schematic illustrating brain states and associated factors distributed in the CSF. Different brain/body states including sleep/wake, appetite, and brain injury and repair have been linked with the presence of distinct factors in the CSF. In the case of brain injury and repair, most of the factors listed have been introduced intraventricularly into injury models, where they were reported to have beneficial effects on recovery from injury. The primary source and mode of action for many CSF factors remain to be elucidated

penetrating incision and reported that IGF2 levels increase within the CSF, peaking at day 7 post-penetrating TBI. Importantly, between 1–7 days post injury, IGF2 protein localizes to the cells within the damaged tissue. Walter et al. also determined that IGFBPs increase in the CSF during that time period, with a predominant increase in IGFBP-2, which is thought to be one of the primary transporters of IGF2 to its target receptor and regulator of its activity [144]. After day 7 post-injury, during the chronic phase of wound recovery, IGF2 levels in the CSF decline with a relative increase in IGFBP-5, thought to be responsible for sequestering and downregulating IGF2 activity, suggesting a possible biphasic response by IGF2 in CNS injury. Throughout adulthood, IGF2 secretion into the CSF may be stimulated by TBI or any injury to the CNS. IGF2 may be an important acute phase protein in TBI for assisting with wound healing through its stimulation of neurogenesis and neuroprotection, whose activity appears to be modulated by concomitant secretion of distinct IGFBPs.

Table 2 Growth factors present in CSF in brain injury

Growth factor	Proposed function in brain injury	Species; injury condition	Reference
BDNF	Neuroprotection	Human; Ischemia	[125]
Erythropoietin	Neuroprotection	Human; Ischemia	[130–132]
IGF-2	Assists with wound healing via stimulation of neurogenesis and tissue homeostasis; possibly involved in promoting phagocytic activity	Rat; Penetrating injury	[115]
NGF	Supports survival and growth of neurons	Human; TBI	[118, 119, 138, 139]
sAPP	Neuroprotection, stimulates proliferation of neural stem cells	Human; TBI	[136, 137]
TGF-β	Supports neuronal survival, suppresses inflammation, regulates glial scar formation, fibrosis, and microglial activation	Human; TBI	[120, 122]
TNF-alpha	Dual role exhibiting both neurotoxic properties in striatum and neuroprotection in hippocampus	Human, Rat; TBI	[133–135]
VEGF	Neuroprotection	Human; TBI	[129]

A biphasic response to CNS injury has also been shown for the hormone-like peptide augurin, encoded by the esophageal cancer related gene-4 (Ecrg4) gene [145, 146]. The Ecrg4 gene has been shown to be a novel tumor suppressor gene [147, 148]. Its protein product, augurin, inhibits proliferation and has been implicated in cell senescence in aged oligodendrocyte precursor cells [149]. In CNS injury, augurin is believed to act as an acute-phase respondent [145, 146]. In studies by Podvin et al. and Gonzalez et al., augurin localizes to the apical membrane of the choroid plexus, is secreted or cleaved from the membrane, and is released into the CSF. Upon penetrating cortical injury in adult rats, Ecrg4 gene expression and augurin immunoreactivity decrease in the choroid plexus 1–3 days post-injury, returning to normal levels by day 7. Importantly, overexpression of the Ecrg4 gene using an adenovirus vector injected into the lateral ventricle leads to decreased bromodeoxyuridine (BrdU) staining along the SVZ of injured rats. Although changes in augurin immunoreactivity in the CSF were not assayed, the authors speculate that augurin undergoes proteolytic cleavage and is released by choroid plexus epithelial cells in response to brain injury. This release depletes augurin from the choroid plexus and CSF, leading to increased cell proliferation for an acute period of time after CNS injury before stores of augurin are repleted by day 7. In this manner, augurin is speculated to participate in the acute phase of wound recovery by dis-inhibiting neural stems cells and promoting their proliferation, a function mediated by CSF-based signaling [145, 146].

Taken together, it appears that one key mechanism for promoting neural repair following TBI is through the endogenous upregulation of neurotrophic factors important in neurogenesis and neuronal survival. An increasing number of growth factors are being discovered in the CSF in response to brain injury and the mechanism of action is slowly being elucidated (Table 2). The CSF may act as a vehicle for volume transmission and transportation of important nutrients and growth factors to sites of brain injury and globally to the CNS to help maintain homeostasis, promote neuroprotection and neurogenesis, and for the removal of toxic by-products generated by the injury.

The potential therapeutics of modulating the CSF in brain injury

Several studies have directly tested the possibility of inducing neurogenesis or promoting neuroprotection by directly infusing known neurogenic growth factors into the ventricles of animal brain injury models. For instance, Sun et al. [150] infused FGF intraventricularly for 1-week post-TBI, which resulted in increased cell proliferation in neurogenic zones of the SVZ and dentate gyrus (DG) and increased survival of newly generated neurons. These favorable effects of FGF correlate with improved water maze testing at 2–25 days post-TBI, suggesting a possible link between increased neurogenesis and memory [150]. Intraventricular administration of FGF2 also has neuroprotective effects in rat models of focal cerebral ischemia.

FGF infusion again increases the numbers of proliferating cells in the SVZ and DG [151], and in some models has actually been reported to decrease infarct size [152]. These observations underscore an interesting connection between FGF2 and CSF since CSF–FGF regulates neurogenesis in the mesencephalon of the developing chick brain [67]. In addition to FGF, intraventricular EGF administration in rats post-TBI (via a fluid percussion injury) also appears to display increased proliferation in SVZ and DG at 1 week post-TBI with improved cognitive functions at 4 weeks post-TBI [153]. Interestingly, NGF infused into the lateral ventricle decreases spatial memory deficits post cortical impact injury as measured by the Morris water maze, an effect suggested to be due to the prevention of cholinergic deficits often seen after TBI [154]. The exact molecular mechanisms mediating these behavioral improvements remain to be elucidated.

Numerous other neurotrophic factors including VEGF, GDNF, and BDNF, have been infused into the CSF to assess their ability to promote neurogenesis and neuroprotection, and to determine if cognitive functioning can be improved following injury [155–161]. In synergistic studies, EGF and erythropoietin infused intraventricularly promote regeneration of damaged cortex and improve motor tasks following focal cerebral ischemia [162]. By a cell-tracking experimental approach using β-galactosidase-labeled SVZ cells, on day 11 post-stroke, 10% of the labeled cells were within the lesioned area, and 55% of the labeled cells were seen migrating from the SVZ. In the same study, on day 18 post injury, 11% of the BrdU-labeled cells in the newly regenerated tissue were also NeuN positive, a marker for neurons, suggesting that a proportion of the newly differentiated neuronal cells in the regenerated cortex appeared to originate from neural stem cells in the forebrain SVZ [162]. This capacity of endogenous neural stem cells to migrate to the site of CNS damage has been demonstrated by other groups as well, and is thought to be mediated in part by the Stem Cell Factor SCF/c-kit pathway [163]. A growing body of evidence in animal models of TBI and stroke suggests that modulating the CSF environment, and therefore creating an enhanced neurogenic and neuroprotective niche, stimulates endogenous neural stem cell proliferation as well as migration, contributing to improved cognitive functions. However, it remains to be seen how effectively these newly generated neurons establish long-term connections in existing brain circuitry.

Although the majority of studies in animal models have been performed with intraventricularly infused growth factors, recent reports suggest that neurotrophic agents can be intranasally administered, with positive effects on the CNS [164–171]. The route for the delivery of growth factors from the nasal epithelium to the CNS is poorly understood. In one model, olfactory and trigeminal nerves transport factors to the olfactory bulb and brainstem, from where they distribute throughout the CNS, including into the CSF [172]. This mode of delivery has been suggested for IGF1 [171]. Intranasal IGF1 has also been suggested to decrease infarct size and improve neurological functioning in animal models of focal ischemic brain injury [173, 174]. Intranasal FGF administered following focal cerebral ischemia in rats has been reported to increase neurogenesis in the SVZ and DG, as well as improve behavioral recovery [165]. Recently in human subjects, it was shown that intranasal insulin penetrates the blood–brain barrier, leading to CSF access [175]. Intranasal insulin was also reported to improve memory and cognitive functioning in patients with mild Alzheimer's disease [166, 169, 170]. Although not all studies assayed for CSF levels of proteins following intranasal administration, many have been shown to be delivered to the CSF, including insulin, exendin, orexin-A, calcitonin gene-related peptide, galanin-like peptide, leptin, melanocortin, and arginine-vasopressin [167].

The possibility of simultaneously administering a cocktail of neurogenic and neuroprotective factors to the CNS provides an intriguing, non-invasive method that is particularly exciting from the therapeutic point of view. The CSF contains a plethora of proteins in addition to growth factors, including proteases, antioxidants, small molecules, and hormones whose function in the CSF is not fully understood [4, 6, 59, 60, 176]. A recent study also showed that media conditioned with choroid plexus supports neural stem cell proliferation compared to media conditioned with cortical tissue [66]. Interestingly, in newborns with hypoxic brain damage, increased levels of antioxidants within the CSF provide neuronal protection [177]. Together, these observations raise the question of whether manipulating endogenous choroid plexus, or injecting or implanting choroid plexus intraventricularly or at the site of brain damage, may provide essential neurogenic growth factors, as well as other proteins necessary to enhance CSF homeostasis. Studies with transplanted choroid plexus in models of focal CNS ischemia have reported reduced infarct size and improved neurological functioning compared to controls [178], with ELISA studies confirming the secretion of GDNF, BDNF, and NGF from the choroid plexus [179]. In these studies, implanted choroid plexus provides essentially an entire cocktail of factors to the injured CNS, enhancing recovery, neuroprotection, and neurogenesis. Although still in its infancy, genetically modifying one's own choroid plexus for secreting distinct peptides or proteins into the CSF offers an intriguing future possibility for CNS therapy [180].

Finally, the CSF is a potential vehicle for volume transmission of implanted neural stem cells, which to date have been used in animal models of spinal cord and brain injury [111, 181–183]. Surprisingly, in an animal model of

Alzheimer's disease in which kainic acid was used to trigger neuronal damage to the hippocampal CA3 region, neural stem cells injected within the ventricles were reported to migrate to the site of injury, differentiate into neurons and astrocytes, and improve cognitive functioning [184]. A similar effect has been observed in an animal model of spinal cord injury, in which neural stem cells injected into the 4th ventricle CSF migrated to the site of injury [181, 182]. A significant limitation to the therapeutic potential is the necessity of intraventricular, intracerebral, or intraspinal injections. It has been suggested that neural stem cells may also be delivered to the CNS intranasally, however the mechanisms underlying these effects remain unclear.

Conclusions

From the early Egyptians to today, the CSF has piqued the curiosity of many. Once thought to control muscle contractions, to carry a certain pneuma or spirit, or to simply be a post-mortem precipitate, the diverse and active roles of the CSF in the developing and adult CNS are finally unfolding. CSF components, which can potentially be dispersed over large areas, may be more significant and pervasive regulators of cortical development, neural stem cell renewal, disease, neurodegeneration, behavior, sleep/wake cycles and other states of consciousness than previously thought. Cells lining the neural tube during development are responsive to CSF signals governing neurogenesis. In the mature brain, cells with access to the ventricles may similarly respond to factors released into the CSF following brain injury, neurodegenerative diseases, aging, as well as daily changes in behavior and activity. The CSF may thus serve as a vehicle for immediate signaling to major control centers of the brain. As such, using the CSF as a therapeutic vehicle to promote CNS homeostasis has many potential benefits. Although the field is still in its infancy, developing strategies to selectively target compounds to the CSF and other areas of the CNS, should open avenues to major therapeutic breakthroughs for TBI, stroke, neurodegeneration, aging, and beyond.

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