The role of motile cilia in the development and physiology of the nervous system

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Motile cilia are miniature, whip-like organelles whose beating generates a directional fluid flow. The flow generated by ciliated epithelia is a subject of great interest, as defective ciliary motility results in severe human diseases called motile ciliopathies. Despite the abundance of motile cilia in diverse organs including the nervous system, their role in organ development and homeostasis remains poorly understood. Recently, much progress has been made regarding the identity of motile ciliated cells and the role of motile-cilia-mediated flow in the development and physiology of the nervous system. In this review, we will discuss these recent advances from sensory organs, specifically the nose and the ear, to the spinal cord and brain ventricles.

1. Introduction

The motile cilium is an evolutionarily conserved organelle. Even unicellular organisms harbour multiple motile cilia (Paramecium & Tetrahymena) or a single pair of flagella (Chlamydomonas) that are structurally similar to vertebrate cilia [1]. The conserved ciliary core, known as the axoneme, consists of nine microtubule doublets that surround a central microtubule pair and is referred to as a 9+2 structure. Ciliary motility is driven by axonemal dyneins, which create sliding interactions between outer microtubules, while other motor proteins, intraflagellar transport proteins, carry cargo into and out of the cilium [1]. Ciliates can interact with their environment in complex ways [2]. Hence, it is not surprising that occasionally sensory components, such as insulin-like receptors [3] are found on the cilium together with other components of signal transduction cascades [4]. In metazoans, besides the motile cilium, another type of sensory cilium exists: the immotile primary cilium. This cilium lacks dynein arms, accounting for its immotility [5], and often the central microtubule pair [6]. Hence, they are referred to as 9+0 cilia. Primary cilia are major signalling hubs [7], exhibiting receptors for serotonin [8], Hedgehog [9], and various odours [10]. The sensory role, however, is not limited to primary cilia, as motile cilia express signalling components too, such as bitter taste-like receptors in respiratory cilia [11] or progesterone receptors in oviductal cilia [12]. Whether cilia harness dynein arms or not is largely determined by specific expression of the Foxj1 transcription factor. This transcription factor alone is sufficient to generate motile cilia [13-15] and is therefore regarded as a marker for motile ciliated cells. Cells, harbouring either a single, two or multiple motile cilia [16], exist in various parts of the nervous system where they generate specific flow patterns.

We will here describe the identity and function of motile ciliated cells in the nose, the ear, the spinal cord, and the brain primarily in animal models used in research and in humans.

2. Cilia in the nose

Chemosensation in vertebrates occurs in dedicated olfactory and gustatory organs. In the nose, bipolar olfactory sensory neurons (OSNs) [17] protrude several olfactory cilia from their dendritic knobs into the nasal cavity and are indispensable to the nasal epithelium across animal species. Indeed, mutations affecting ciliogenesis or intraflagellar transport of transduction components into the olfactory cilia [10, 18] result in anosmia in humans, mice [19] and zebrafish [20]. In addition, the olfactory epithelium contains microvillous odorant receptor cells, glia-like support cells (sustentacular cells), and basal cells which replenish the OSNs. The anatomically separated respiratory epithelium consists of mucus-producing goblet cells and multiciliated support cells [21-24]. This arrangement is observed in all vertebrates and has been documented for humans [23], mice [24], clawed frogs [21] and zebrafish [22] (Figure 1 and 2a). Nevertheless, vertebrate olfactory organs are highly variable. Fish exhibit an aquatic nose, mammals an airborne nose, and amphibians a combination of the two [17]. Despite vast differences in their environment, odorant receptors are conserved between aquatic and terrestrial animals [17] and localize specifically to the cilia of the OSNs [10]. Olfactory cilia lack dynein arms in many species including humans [23], rodents [25] and zebrafish [22], and are therefore considered immotile. Yet, OSNs express the motile ciliary marker *Foxj1* in mice [26, 27] and olfactory placodes express *foxj1b* in zebrafish [28, 29]. Strikingly, olfactory cilia were observed to be motile in frog [30] and trout [31]. The motile nature of those cilia, however, remains puzzling.

Nonetheless, multiciliated cells are found near the olfactory epithelium in many species (Figure 2a). Their function, however, remains poorly understood. Motile cilia may remove pathogens entrapped in mucus away from the olfactory epithelium [37], in a process similar to the mucociliary clearance of the lung epithelium [38]. Olfactory cilia are the single direct entry point into the central nervous system from the outside [39] and are specifically targeted by pathogens [40]. Thus, mucociliary clearance [38] may complement other defence mechanisms including enzymatic activity targeting pathogens [41] and sneeze-reflexes [42].

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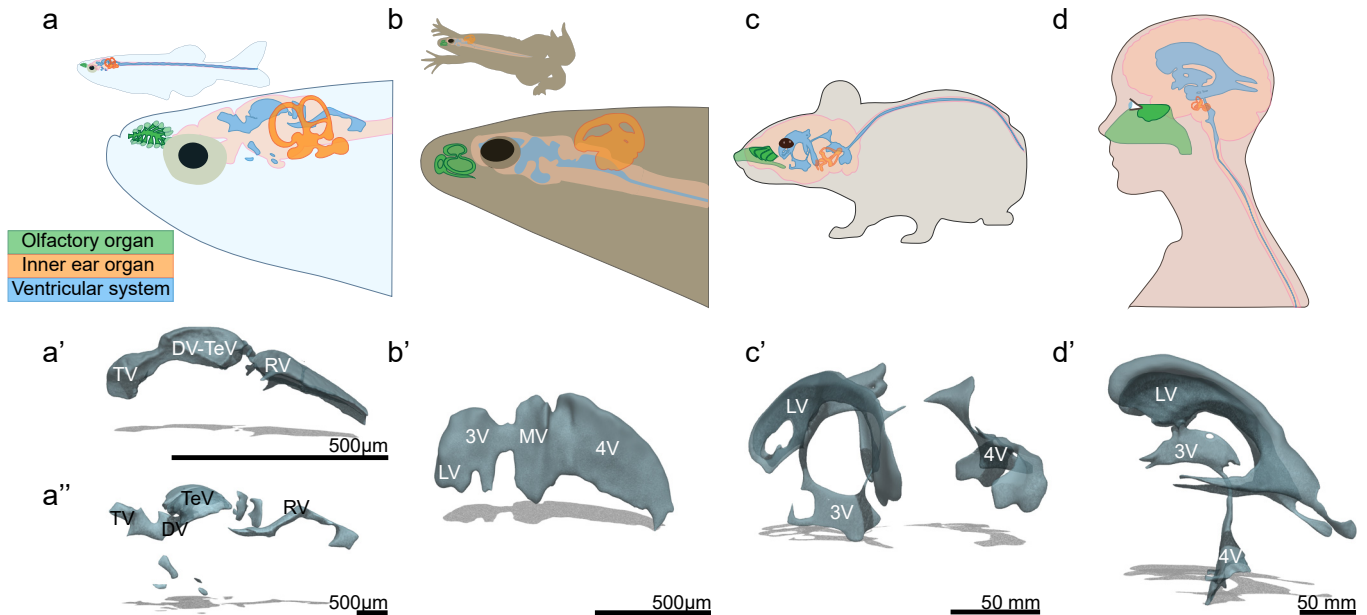
Figure 1. Schematic depiction of sensory and ventricular systems across vertebrates.
(a-d) Olfactory organs (green), inner ears (orange), ventricular systems (blue) and central nervous systems (pink) in zebrafish (a), frog (b), mouse (c) and human (d) are shown. Sensory regions in olfactory organs are coloured dark green, while non-sensory regions are light green. Three-dimensional renderings of brain ventricular systems of (a') a two-day old zebrafish [32], (a'') a three-month-old zebrafish [33], (b') a stage 45 Xenopus tropicalis [34], (c') an average adult mouse [35], and (d') an adult human [36] are shown. TV = telencephalic; DV = diencephalic ventricle; TeV = tectal ventricle; RV = rhombencephalic ventricle; LV = Lateral ventricle; 3V = 3rd ventricle; MV = mesencephalic ventricle; 4V = 4th ventricle.

Additionally, motile-cilia-mediated flow may contribute to odour sampling in the nose. In the aquatic environment, odour molecules flow through the olfactory organ, either by diffusion or active mechanisms including motile cilia [43-45]. Motile cilia do not only attract odorants into the nasal cavity to aid odour detection, but simultaneously repel odours to enhance detection of rapidly succeeding odour plumes in the zebrafish nose pit (Figure 2a) [44]. Cilia-mediated flow may also support the sequential enzymatic conversion and delivery of odorants, such as ATP, to the nose [45]. For mammals and other terrestrials, such as insects, volatile odours first need to reach OSNs through diffusion or active transport into the mucus or fluid surrounding OSNs [17]. In mammals, sniffing does not only aid odorant transport to the olfactory epithelium, but also induces sniff cycle-related temporal dynamics in the olfactory bulb that facilitate odor coding [46, 47]. Such a process may be mediated by mechanosensitivity of olfactory cilia [48]. To further aid phase transition of odorants, both mammals and insects express odorant binding proteins, which travel freely in the mucus and help capture volatile odours into the mucus layer close to the sensory cilia [41, 49]. It remains unclear whether motile-cilia-mediated mucus flow in the nasal cavity of mammals contributes to the clearance of odours, and thereby plays similar roles as in fish.

3. Cilia in the inner ear
Whereas OSNs are key to olfaction, hair cells are essential to auditory and vestibular processing[50]. The hair cell bundle consists of multiple tapering stereocilia and sometimes a single kinocilium, the only true cilium. Both vestibular and cochlear kinocilia do not directly transduce sensory information. Even more so, hair cells in the cochlea of anamniotes lose their cilium during the maturation process[50]. Instead, gated ion channels on stereocilia open upon deflection of the hair bundle and initiate a cellular response. Hair cells mediate stimulus detection of head rotation in the semi-circular canals, linear acceleration in the otolithic organs, and sound in the otolithic organs of fish or amphibians, and in the cochlea in mammals [52].

In mammals, otolithic kinocilia lack inner dynein arms [53] and are thus considered immotile. Similarly, kinocilia in the zebrafish inner ear are considered to be immotile [51, 54-57], despite an initial report stating otherwise [58]. Surprisingly, oscillating kinocilia have been observed in the otolith organ of eels [59]. Despite the immotility of the kinocilium, hair cells express the motile cilia marker *foxj1b* in zebrafish [28, 54], *foxj1.2* in the clawed frog otic vesicle, [60, 61], and *Foxj1* in mice in the cochlea prenatally [62], and in the utricle both pre- and postnatally [62, 63]. Interestingly, motile cilia in auditory organs have been found elsewhere. For instance, the chordotonal sensory neurons of *Drosophila* bear cilia, whose motility is shown to amplify environmental sounds [64], such that mutants lacking ciliary motility are deaf [65]. In contrast, mammalian hair cells do not rely on kinocilia to amplify environmental sounds. Instead, this role is attributed to an active piezo-element in the hair cell membrane [52]. Beating cilia have also been observed in the zebrafish otic vesicle during early development, specifically on cells neighbouring hair cells [54-58] (Figure 2b). Besides clear evidence of ciliary motility, there is no consensus on the timely location [54-58], the ciliary beat frequency [55-58], or the consequences of ciliary immotility on otolith formation. Even though ablation of ciliary motility in zebrafish affects the otolithic number [54, 58] and shape [54, 55], these phenotypes disappear at later developmental stages [56, 57]. It is possible that other

mechanisms regulate the later development of the ear. Bodily movements may be involved in this process since restraining larval movements perturbs otolith development [56, 66] and even aggravates the motile-cilia-mediated ear phenotype [57]. Even though the otolith phenotype disappears over time, the young zebrafish larvae ay depend on motile-ciliated flow for its inner ear function at early age. Since otolith size affects auditory perception [67] and vestibular processing [68], defects in otolith formation may result in an imbalance that compromises larval zebrafish survival [68]. In fish, otoliths are directly tethered to kinocilia, while in amniotes, multiple hair cells are covered by an otoconia-covered membrane. Little is known, however, about the presence or absence of motile cilia in amniotes. Although otolith formation in fish may not directly translate to mammals, understanding cilia-mediated control, as well as the importance of *Foxj1* expression, could provide important mechanistic insights into ear development.

4. Motile cilia in the spinal cord
Motile cilia are observed on several cell types in the spinal cord, including floor plate cells, ependymal cells and cerebrospinal fluid-contacting neurons (CSF-cNs) (Figure 2c). These cells are adjacent to the central canal, which elongates from the brain ventricles throughout the entire spinal cord and is filled with cerebrospinal fluid (CSF) [69, 70].

4.1 Identity of motile ciliated cells in the spinal cord
The floor plate is present during early development in all vertebrates and consists of cells populating the ventral midline of the neural tube. Through the secretion of Sonic hedgehog, floor plate cells play key roles in the patterning of the neural tube [71]. Work in various animal models including zebrafish [13], mice and chick [72], revealed that the floor plate cells harbour cilia and express typical markers of cilia motility including *Foxj1*. Moreover, ciliary motility was observed in zebrafish floor plate cells [73-75], but remains to be investigated in other species.
Ependymal cells of the central canal (ECCs), which commonly refer to the cells directly contacting the central canal [76], also harbour motile cilia (Figure 2c). ECCs primarily originate from the ventral progenitor domains of the neural tube during spine development [77-79] and retain the ability to proliferate at postnatal stages. Most proliferation occurs either during spinal cord growth [80, 81] or upon spinal cord injury [76, 82, 83]. ECCs have been observed in all analyzed vertebrate central canals and possess fewer cilia than ependymal cells of the brain. In fish and amphibians, ECCs have commonly been referred to as ependymo-radial glial cells (ERG) [84]. In fish, birds, amphibians and reptilians, ECCs harbour one and sometimes two cilia [83, 85], while in mice, rat and guinea pig, ECCs are bi-ciliated [80, 85, 86], or occasionally bear up to 3-4 cilia in multinucleated cells [80, 82]. In larger mammals, such as rabbits [85], macaques [81] and humans [81], two populations of ECCs with either 1-2 or 20-30 cilia co-exist and are spatially organized; multiciliated cells are located laterally, while mono- and bi-ciliated cells are situated ventrally and dorsally [81]. This suggests that the number of cilia on ECCs correlate with the size of the spinal cord and central canal [85]. Interestingly, only bi-

ciliated cells were shown to proliferate [81].
CSF-cNs, also known as Kolmer-Agdhur cells , are the third motile ciliated cell type in the spinal cord. They primarily constitute GABAergic and PKD2L1-positive neurons located at the interface between the nervous system and the CSF [69, 87-90]. Two populations of CSF-cNs co-exist; the dorsal CSF-cN' and the ventral CSF-cN'', which emerge from different progenitor domains during early spinal cord development [69, 87, 91-94]. The morphology of CSF-cNs is peculiar. They display an apical dendritic extension directed towards the central canal, protruding a tuft of microvilli [69, 90, 95]. CSF-cNs possess a cilium in clawed frog [96, 97], lamprey [88, 98, 99], zebrafish [100], chick [101], and turtle [102], and the motility of this cilium was confirmed in lamprey and zebrafish [88, 99, 100] (Figure 2c). However, there is no consensus on whether a cilium exists on CSF-cNs in mammals [69]. Considering their particular morphology and resemblance to hair cells, CSF-cNs were suggested to be sensory neurons integrating mechanosensory and chemosensory cues from the CSF [69]. Recent evidence in zebrafish and lamprey confirmed that CSF-cNs are mechanosensory [74, 99, 100]. CSF-cNs respond to both the continuous CSF flow present in the central canal and bending of the tail through the specific expression of PKD2L1 [74, 100], a channel previously implicated in flow sensation [103, 104]. CSF-cNs also detect pH changes in the CSF through acid-sensing ion channels [99] and PKD2L1 [89] in mice and lamprey. CSF-cNs were shown to maintain spine morphology [74] and modulate locomotion [100] in zebrafish. Yet, the importance of motile cilia in CSF-cNs physiology remains poorly understood. Sternberg et al. observed that the response of CSF-cNs to muscle contractions was reduced in absence of ciliary motility, although PKD2L1 correctly localizes to the apical extensions of CSF-cNs [74]. These results suggest that the motile cilium of CSF-cNs may contribute to the sensory function, but the precise mechanisms remain to be discovered.

4.2 Functions of ciliary beating in the development and maintenance of the spine
In agreement with the observations of ciliary motility in the spinal cord, movement of CSF occurs along the central canal. This is well described in zebrafish from 24 hours of development, when most of the motile cilia are located on the ventral part of the central canal and generate a bidirectional flow, moving caudally along the ventral wall and rostrally along the dorsal wall [74, 75, 105] (Figure 2c).
Work in zebrafish has provided many insights regarding the function of motile cilia in spine development. Ciliary motility is essential for the straightening of the body axis at early developmental stages in zebrafish. ENU mutagenesis screens were the first to describe zebrafish mutants with a curly tail phenotype [106], which has since then, been ascribed to motile ciliary defects [107]. This phenotype is only recently being understood. First, ciliary motility and CSF flow are crucial to form the Reissner's fibre [75], which is an extracellular thread primarily composed of the glycoprotein SCO-spondin secreted by the floor plate and the sub-commissural organ [108]. In turn, the Reissner's fibre is needed for the straightening of the body axis, in a process independent from CSF flow or cilia motility,

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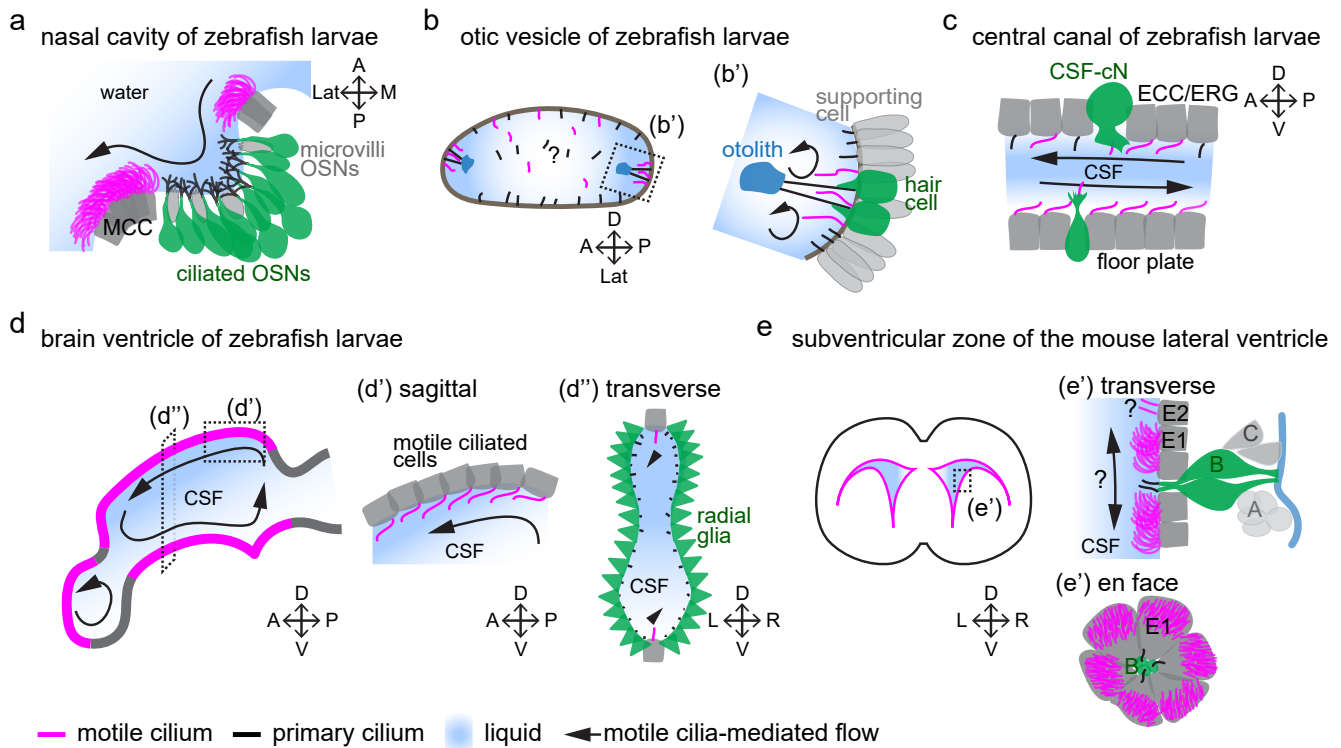
Figure 2. Schematic depiction of various cavities of the nervous system lined with motile cilia.
(a) The olfactory organ of a zebrafish larvae is composed of multiciliated cells (MCC) located at the outer rim of the nasal cavity. MCC bear multiple motile cilia (magenta), which generate a directional fluid flow of water. Ciliated olfactory sensory neurons (OSNs, green), which bear multiple primary cilia (black) and microvilli OSN (grey) are located at the bottom of the nasal cavity. (b) The otic vesicle of zebrafish embryo at 18-24hpf contains hair cells (green), or tether cells, that bear primary cilia capable of tethering the otolith (blue). (b') Next to hair cells, there are motile cilia on supporting cells that generate a rotational flow near the otolith. (c) The central canal of the spinal cord is composed of CSF-cNs (green), which bear a microvilli tuft and a motile cilium in zebrafish. Ependymal cell of the central canal (ECC, grey), also known as ependymo-radial glia cells (ERG) in zebrafish, are located on the floor plate or the dorsal wall of the central canal and bear a cilium. Note that there are more motile cilia in the ventral part of the central canal than the dorsal plane at early developmental stage, and that the CSF flow is bidirectional. (d) The brain ventricular system of the zebrafish larvae is decorated by motile cilia (magenta) at very specific location along the midline. Motile cilia-mediated flow is complex and compartmentalized to individual ventricles. (d') Sagittal view of the inset in (d) showing that cells bear a single cilium oriented anteriorly in the same direction as fluid flow. (d'') Transverse view shows that motile cilia are located in the ventral and dorsal wall of the diencephalic-tectal ventricle. Elsewhere, radial glia (RG, green) project their primary cilium into the CSF-filled cavity. (e) Ependymal cells, which bear motile cilia, are located along the medial and lateral wall of the mouse lateral ventricle. (e') Transverse section through the inset in (e) reveals that neural stem cells of the subventricular zone are located directly under the ependyma layer made of multiciliated E1 cells and bi-ciliated E2 cells. Neural stem cells also known as B cells (green) project their primary cilium toward the CSF-filled ventricle in addition to contacting the blood vessel (blue), while transient amplifying cells (C cells, grey) and migrating neuroblasts (A cells, grey) lose their direct interaction with the CSF. En face representation shows the pinwheel structure composed of E1 and B cells. Note the translational polarity of the motile cilia of E1 cells. A: anterior, P: posterior, L: left, R: right, D: dorsal, V: ventral, M: medial, Lat: lateral. Motile cilia are in magenta, primary cilia are in black.

which is poorly understood [75]. Second, motile-cilia-mediated transport of molecules from the brain to the spinal cord [73, 74] controls spine development. Brain-derived adrenaline induces the release of the urotensin peptides URP1 and URP2 by spinal CSF-cNs, which act on the muscles of the developing embryos to straighten the body [109]. Surprisingly, no other animal model but the zebrafish shows such striking developmental defects upon loss of ciliary motility in the early neural tube [72].

Later in development, CSF flow and ciliary beating help maintain a straight body axis in zebrafish. Inhibition of motile cilia function at post-embryonic stages reveals a high incidence of scoliosis [33, 110, 111]. In addition, a zebrafish model of scoliosis carrying a mutation in the *ptk7* gene shows defects in cilia and ciliary flow [110, 112] even before the appearance of spinal curvature [113]. The mechanisms linking scoliosis, ciliary motility and CSF flow are still poorly understood. Neuroinflammation may be responsible, as proinflammatory signals are sufficient to induce scoliosis-like spinal curvature, and treatment with immunomodulating therapies reduces the severity of scoliosis [113]. Next, the abovementioned adrenaline-urotensin signalling may be involved in this phenotype, since urotensin receptor *uts2ra* mutant zebrafish develop scoliosis [109]. Interestingly, rescuing the expression of the scoliosis-associated gene *ptk7* solely in the motile ciliated cells of the brain ventricles is sufficient to rescue the scoliosis phenotype [110, 113]. This suggests that brain-released factors travel to the central canal and maintain the straight body axis, but the precise molecular mechanisms remain unknown. Most studies on the importance of cilia-mediated flow have been performed on zebrafish, and some studies support a conserved role for CSF flow in spine development in mammals. For instance, developmental scoliosis is observed in human conditions associated with perturbations of CSF flow, including neural tube closure, spinal canal cyst and Chiari malformation [114]. Nonetheless, stenosis of the human central canal has been observed in the healthy population after the age of 10 years, yet it remains a subject of debate

and raises questions to the function of the central canal in adult human physiology [81, 115, 116].

5. *Motile cilia in the brain ventricular system*

Motile cilia are also found within the brain ventricular system, which is the conserved complex of CSF-filled cavities in the brain. Here, CSF is circulated throughout the brain ventricular system to nourish the brain, maintain brain homeostasis and support neurogenesis[117-121]. One major contributor to such CSF flow is the motile cilia of the ependymal cells (ECs) lining the ventricles [122, 123].

5.1 *Development and cellular composition of the brain ventricular system*

The embryonic brain vesicles, which later develop into the brain ventricular system, are remarkably conserved across vertebrates. Initially, the hollow neural tube bends to generate three fluid-filled cavities, one in the telencephalon, one in the diencephalon and one in the rhombencephalon, akin to the three ventricular cavities of the larval zebrafish (Figure 1) [124, 125]. As the brain further develops to its adult anatomy, the telencephalic ventricle transforms into two lateral ventricles in amphibians and mammals, but not in zebrafish, such that the mature ventricular system constitutes four cavities (Figure 1) [33, 124, 125]. Furthermore, the telencephalic ventricle in teleost fish is located dorsally above the brain parenchyma, in contrast to the deeply embedded ventricles of other vertebrates [126]. This is likely due to the unique telencephalic morphogenesis of teleosts, wherein the tissue everts and folds outwards [126], contrasting the telencephalic evagination of other vertebrates.

Already in 1836, the neuroanatomist Purkinje described ciliary beating on cells along the sheep ventricles [127]. Since then, these cells, referred to as ependymal cells (ECs), have been described in both fish [32, 110, 128-130], amphibians [131-133] and mammals [16, 123]. Traditionally, the ECs of the brain are defined as *Foxj1*-positive, motile ciliated, cuboidal cells generating near-wall CSF flow [16, 32, 133-136]. In mammals, the multiciliated ependymal lining appears during late prenatal and early postnatal stages [137-141], even though ECs are already committed during embryonic development [138, 141, 142]. ECs derive from embryonic radial glial cells, which are neural stem cells (NSCs) generating neurons [143], glia [138, 142, 144], as well as the NSCs (termed B cells) of the adult neurogenic subventricular zone (SVZ) [141, 142]. Furthermore, the ECs and adult NSCs share a subpopulation of radial glia as their common progenitors [141, 142]. In contrast to ECs of the spinal cord that can proliferate postnatally, ECs in the mammalian brain are considered to be post-mitotic [136, 142]. Yet, this is still debated, as some studies suggest ECs may dedifferentiate and proliferate [145-147]. Like mammals, both zebrafish and the clawed frog have a multiciliated ventricular lining in adult stages, despite the presence of motile monociliated cells in larvae [32, 129, 130, 133, 148] (Figure 2d). Interestingly, although most of the adult ventricular lining of rodents consists of multiciliated cells, even in the mouse, mono- and biciliated cells do exist [149, 150] (Figure 2e). Since these cells contact the ventricular lumen and extend long radial processes into the neuropil, they are thought to be tanycytes relaying chemical and mechanical information from the CSF to the underlying neurons [150]. In addition to the important role of ECs in circulating CSF, studies also suggest that ECs secrete molecules into the fluid [151], and thus relay signals from the neural tissue to the CSF. Nevertheless, the main contributors to the CSF contents in adult vertebrates are the choroid plexuses [152, 153]. These structures, which exist in each ventricle in mammals, consist of specialized epithelial cells, transporting ions and water from blood capillaries to the ventricular lumen. Furthermore, the choroid plexus cells themselves produce and secrete many proteins into the CSF [154]. As such, the choroid plexuses make up a barrier between the blood and the CSF, tightly controlling the CSF content. Interestingly, the choroid plexus cells exhibit cilia, which are motile in zebrafish [155], but mostly immotile in mice [156]. The function of these cilia is not fully understood, yet in zebrafish, they may contribute to CSF flow [155], while in mice they are suggested to serve a chemosensory function [156].

5.2 *Regulation of the CSF flow*

The flow of CSF within the ventricular system, which is contributed by multiple factors in addition to cilia, is complex. Moreover, the properties of CSF flow vary with the proximity to the ventricular walls, following a principle known as boundary layers [135]. Therefore, the description of CSF flow is commonly separated into two major levels; the macrofluidic, bulk flow amid the ventricular cavities, and the microfluidic, near-wall flow contributed by the ECs [135]. In mammals, the bulk CSF flow emerges at the secretion sites (the choroid plexuses), and move through the third and fourth ventricles into the subarachnoid space, wherein it escapes the brain ventricular system [157-159]. This overall bulk, unidirectional flow is suggested to arise from several sources, like the pressure gradient caused by CSF secretion and exchange of CSF for interstitial fluid across the ependymal lining [138]. Pressure changes may also be contributed by the cardiac [135, 160] and respiratory cycles [160, 161]. Interestingly, bodily movement temporally changes the direction of CSF flow in humans [162] and in the zebrafish brain ventricular system [32]. Since many physiological parameters impact the bulk flow, and are difficult to measure with high spatial and temporal resolution, most studies focus on the cilia-mediated flow along the ventricular walls. The contribution of motile cilia in CSF flow is clearly demonstrated in zebrafish [32, 110], clawed frog [34, 133], rodents [e.g. 123, 137, 139, 163, 164], pigs [163] and humans [123]. Notably, the cilia-mediated, near-wall flow is complex, wherein local domains of cilia-generated currents may serve to target certain molecules to specific areas [32, 163] (Figure 2d). It is well documented that such cilia-mediated flow is crucial to maintain a properly functioning brain ventricular system, as zebrafish, clawed frog and mouse ciliary mutants display ventricular defects eventually causing hydrocephalus [e.g. 32-34, 110, 111, 133, 137, 139, 164]. Surprisingly, human patients with primary ciliary dyskinesia rarely develop hydrocephalus [165]. This observation poses the question as to whether the relative importance of near-wall cilia-mediated flow and bulk flow differs across species. The ventricular sizes may also influence the importance of bulk compared to

near-wall flow, and advocates the continued use of several animal models to disentangle the CSF flow patterns. Such animal models will also be pivotal to understand how the CSF flow patterns are regulated. A few studies revealed that neural states may impact the ciliary beating of ECs. For instance, the CSF flow patterns change in the third ventricle of mice during the day versus the night [163]. Furthermore, neuropeptides like melanin-concentrating hormone, may increase the ciliary beating frequency of the ECs [166]. Yet, the influence of such local changes in ciliary beating on the global CSF flow remains poorly understood.

5.3 Functions of the CSF flow

The differential nature of the bulk flow and the near-wall flow suggests these different levels of CSF flow may serve different means of relaying signalling and developmental cues. It is likely that the bulk mid-ventricular flow supports brain homeostasis and volume transmission, which is the long-range, intercellular communication [167, 168]. Transfer of neuropeptides across brain ventricles was shown to not only promote basic physiological needs like hunger [169], but also increase neuronal excitability to enhance cortical alertness in response to acute stress [151]. Considering the apposition of the embryonic and adult neurogenic zone to the ventricles (Figure 2d, 2e), it is highly probable that CSF flow supports the neurogenic capacity of NSCs through the specific delivery of chemical or mechanical cues. Interestingly, the CSF proteome is regionalized due to the differential transcriptome of the choroid plexuses in the various brain regions in mouse embryos [154] and also changes substantially from early to late embryonic stages [170] and during ageing[153]. These regional and temporal changes in the CSF composition have a direct impact on the cell fate and proliferation rate of the neurogenic tissue apposing the CSF [153, 154, 170]. Furthermore, these secreted signals can be rather specific. For instance, WNT5A, secreted by the hindbrain choroid plexuses, travels to distinct neural progenitors within the developing hindbrain to support cerebellar development [171]. Altogether, these findings indicate that CSF flow promotes regionalization of the CSF contents as proposed in larval zebrafish [32] (Figure 2d). Other developmentally important CSF-borne signals, like Igf-2 [117], may be localized to specific areas by the near-wall flow patterns. Whether this supports the distinctive differentiation of brain regions remains to be investigated.

While the neurogenic capacity is largely retained in teleosts and amphibians throughout adulthood [84, 148, 172], mammalian neurogenesis is confined to two brain regions, namely the SVZ [173, 174], located just beneath the ependyma of the lateral ventricle, and the subgranular zone of the hippocampal dentate gyrus [175]. In mice, NSCs located in the SVZ extend a primary, immotile cilium into the ventricles (Figure 2e). This apical extension is surrounded by ECs to form so-called "pinwheels" [149]. Since primary cilia display a multitude of mechano- and chemosensory receptors [6, 7, 176], cilia of the neural progenitors may integrate cues from the CSF flow and regulate neurogenesis. In the embryonic and early postnatal stages in mice, the primary cilia of radial glia express the mechanosensors PKD1 and PKD2 [177], which not only regulate the polarity of multiciliated

ECs [177], but also promote the differentiation of radial glial cells to neurons [121]. The primary cilia of NSCs may play similar functions in adults. Indeed, ablation of primary cilia in a subpopulation of SVZ NSCs resulted in reduced neurogenesis [118]. Furthermore, a study in mice demonstrated that applying mechanical forces onto the ventricle-contacting, apical domain of adult NSCs in the SVZ, promoted neuronal proliferation through the flow-sensing epithelial sodium channel [119] in a cilia-independent manner. However, the nature of the incoming signal to the NSCs remains poorly understood and may also be chemical. Studies have demonstrated that the binding of developmental signalling cues to receptors on the NSC primary cilia may promote proliferation [117] or maintain cellular quiescence [120]. While these studies suggest that CSF flow transports molecules to the SVZ in the lateral ventricle, CSF flow may also support a more fine-tuned distribution of molecules into gradients. For instance, Sawamoto et al. [178] showed that the distribution of the chemorepellent Slit2 may drive proper neuroblast migration from the SVZ towards the olfactory bulb.

6 Conclusion

In summary, motile cilia serve a variety of functions within the nervous system. There is now clear evidence for the role of these organelles in circulating fluid, both in sensory systems like the nose and ear, in the spinal cord, and in the brain ventricles during organ development and homeostasis. It remains however less understood how motile cilia allow the nervous system to sample its environement, whether it is achieved through the establishement of chemical gradients, delivery of molecules to precise targets or mechanical forces. Moreover, it is remarkable that the nervous system may manipulate ciliary beating, yet the extend and impact of such regulation on the development and physiology of the brain remain to be studied. Investigations across systems, species and developmental stages will now be pivotal to further disentangle this mutual dependence of the nervous system on proper motile cilia functioning. Ultimately, such studies will be essential to understand and develop treatments for disorders associated with ciliary defects, like scoliosis and hydrocephalus.

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