Cerebrospinal fluid-contacting neurons: multimodal cells with diverse roles in the CNS

Claire Wyart, Martin Carbo-Tano, Yasmine Cantaut-Belarif, Adeline Orts-Del'immagine, Urs L Böhm

Cerebrospinal fluid-contacting neurons: multimodal cells with diverse roles in the CNS

Claire Wyart[1†], Martin Carbo-Tano[1], Yasmine Cantaut-Belarif[1], Adeline Orts-Del'Immagine[1] and Urs L. Böhm[2]

[1] Institut du Cerveau (ICM), INSERM U1127, UMR CNRS 7225 Paris, Sorbonne Université, Paris, France.
[2] NeuroCure Cluster of Excellence, Charité Universitätsmedizin Berlin, Berlin, Germany.

[†]email: claire.wyart@icm-institute.org

Abstract| The cerebrospinal fluid (CSF) is a complex solution that circulates around the CNS, and whose composition changes as a function of an animal's physiological state. A type of ciliated neurons that are bathed in the CSF — and thus referred to as CSF-contacting neurons (CSF-cNs) are unusual polymodal interoceptive neurons. As chemoreceptors, CSF-cNs respond to variations in pH and osmolarity and to bacterial metabolites in the CSF. Their activation during infections of the CNS results in secretion of compounds to enhance host survival. As mechanosensory neurons, CSF-cNs operate together with an extracellular proteinaceous polymer known as the Reissner fibre to detect compression during spinal curvature. Once activated, CSF-cNs inhibit motor neurons, premotor excitatory neurons and command neurons to enhance movement speed and stabilize posture. At longer timescales, CSF-cNs instruct morphogenesis throughout life via the release of neuropeptides that act over long distances on skeletal muscle. Finally, recent evidence suggests that mouse CSF-cNs may act as neural stem cells in the spinal cord, inspiring new paths of investigation for repair after injury.

[H1] Introduction

The cerebrospinal fluid (CSF) is a complex biological solution that is largely produced by the choroid plexuses **[G]** and can change composition as a function of age or physiological state (**Box 1**). The CSF circulates in the brain ventricles, in the central canal in the spinal cord and in the subarachnoidal spaces surrounding the CNS[1] (**Fig. 1a**). A large body of work in the last decade has shown that, at the interface between the CNS and the CSF, ciliated CSF-contacting neurons (CSF-cNs) act together with the Reissner fibre (a long acellular proteinaceous thread present in the fourth ventricle and central canal) as an axial mechanosensory system that can detect spinal curvature[2–7]. Upon activation, spinal CSF-cNs modulate the activity of motor neurons and premotor excitatory interneurons in the hindbrain and spinal cord, as well as the output of reticulospinal neurons in the hindbrain projecting down to spinal cord. The discovery of an axial mechanosensory system in the vertebrate spinal cord challenged the classical view that the sensory modulation of posture and locomotion relies only on proprioceptive **[G]** information carried by dorsal root ganglia neurons located in the peripheral nervous system[8] (**Fig. 1b,c**). It is now clear that, in addition to this mechanosensory function, CSF-cNs can act as chemosensors that detect molecules in the CSF[9–13] and, in turn, regulate the CSF composition via active neurosecretion[11,12,14–22]. The polymodal interoceptive **[G]** system mediated by CSF-cNs therefore provides a novel means to mediate brain–body interactions by linking together the CSF, the nervous system and effectors in other systems and organs distributed throughout the body.

In this Review, we will outline the established roles of the axial sensory system contacting the CSF in motor control, morphogenesis and innate immunity **[G]**. A major limitation faced by those investigating the functions of this sensory system in vivo has been the difficulty in accessing CSF-cNs, which are nested in the center of the spinal cord. However, in the last decade and thanks to the transparency and genetic accessibility of zebrafish at early stages of development, this challenge has been overcome, opening new paths of investigation and revealing numerous physiological functions for this polymodal axial sensory system. Consequently, most of the work reviewed in this article (and unless otherwise stated) comes

from experiments performed in zebrafish. Investigations performed in other animal models, such as mice, lamprey and macaques, are highlighted where appropriate.

[H1] Features of CSF-contacting neurons

[H2] CSF-cN anatomical localization and morphology

A century ago, Kolmer and Agdur analyzed the spinal cords of over a hundred vertebrate species and described the morphology of a type of ciliated cells that were in contact with the CSF and that reminded them of hair cells, the primary inner ear sensory receptor cells that transduce mechanical stimuli evoked by sound into electrical signals transmitted to the brain[3,23–25]. These cells have since been called spinal CSF-cNs or Kolmer–Agduhr cells[23,26–37]. Throughout vertebrate species, CSF-cNs are inserted in the ependymal layer that surrounds the central canal of the spinal cord and exhibit a peculiar morphology: their apical extension bathes in the CSF and is in close vicinity to an acellular proteinaceous thread called the Reissner fibre that is formed by the aggregation of the large protein SCO-spondin (**Fig. 1c; Box 1**). Because the ciliated apical extension of the CSF-cNs resembled the extensions present on hair cells, Kolmer and Agduhr postulated that these neurons constitute a sensory system, which they termed a "parasagittal organ", that could detect changes of properties of the CSF. Kolmer even suggested that this sensory system could constitute a "third ear in the spinal cord" and proposed that CSF-cNs and the Reissner fibre interact to form a mechanosensory system[24]. Due to the variability in the presence and location of the Reissner fibre after dissection and fixation of the spinal cord, however, Agduhr doubted that the Reissner fibre could contribute to the sensory function of CSF-cNs[25]. A century later, the expansion of gene-targeting methods and optical tools for monitoring and manipulating the activity of CSF-cNs in vivo in transparent model organisms such as larval zebrafish has enabled researchers to resolve this controversy.

Across vertebrates, the soma of CSF-cNs is inserted in or immediately under the layer of ependymal cells that surrounds the central canal[38,39]. All spinal CSF-cNs are marked by the

expression of polycystic kidney disease 2-like 1 (PKD2L1) channels, but one subpopulation is located in a dorsolateral position, relative to the central canal, while the other is found ventral to the central canal[38,40–42]. In mice[43] and zebrafish[27,31,35,41], these two populations of CSF-cNs originate from different progenitor domains **[G]** (see below).

There are two peculiarities of spinal CSF-cN morphology compared to that of hair cells (**Box 2**): first, CSF-cNs are not excitatory cells that release glutamate as a neurotransmitter, but GABAergic neurons[44,45] with an axon ascending in the spinal cord (and, in the case of the most rostral CSF-cNs, in the hindbrain [46]). Second, CSF-cNs exhibit an apical extension protruding from their soma that contains one cilium **[G]** and multiple microvilli **[G]** that contact the CSF [14,21,24,25] and do not form an organized structure such as the staircase formed by stereocilia in hair cells. Their apical extension has a dendritic origin (being labelled by the dendritic marker microtubule-associated protein 2 (MAP2))[47]. The CSF-cN cilium contains microtubules **[G]** (as identified in zebrafish[4,42], xenopus[26], rats and mice[44,48]) that are surrounded by numerous seemingly-disorganized actin-based microvilli, whose structure varies among species. Ultrastructural investigation of the apical extension of ventral CSF-cNs in the sagittal plane[42] shows that the cilium typically has a 9+2 arrangement of microtubules, with nine doublets surrounding a central pair of singlets — a hallmark of a motile cilium (often referred to as a kinocilium **[G]** [4]). Accordingly, this single cilium has been shown to be motile in the lumen of the central canal in vivo (**Supplementary Video 1**) and in primary cultures of CSF-cNs[4,42] (**Supplementary Video 2**). Evidence for a subset of dorsolateral CSF-cNs exhibiting a cilium with a 9+0 structure has been reported in lamprey using super resolution microscopy combined with light-sheet imaging[12], suggesting that the cilium of dorsolateral CSF-cNs may not be motile. Although the 3D organization of microvilli in the CSF-cN apical extension differs across species, it is in all cases characterized by an elongated neck linking the cell body to the apical pole[38]. Ultrastructural examinations of the central canal showed that the luminal pole of CSF-cNs is in close vicinity to the Reissner fibre[7] (**Supplementary Video 3**). While the Reissner fibre and CSF-cNs appear to be conserved in numerous vertebrate species, including zebrafish, mouse and macaques[38] (no investigations have yet been reported in humans), the diameter of the fibre and the peculiar organization of the microvilli in the CSF-cN apical extension vary across species (as

an example, the diameter of the Reissner fibre is 250 nm in larval zebrafish[7] versus 50 μm in cows[49]).

[H2] CSF-cN molecular markers

The discovery of a specific genetic marker for spinal CSF-cNs unexpectedly came from the investigation of a channel expressed in taste buds and necessary for sour taste in mice[9]. Polycystic kidney disease 2-like 1 (PKD2L1)[9] is a non-selective cationic channel that, together with the transmembrane protein polycystic kidney disease protein 1-like 2 (PKD1L2), is specifically expressed in spinal CSF-cNs in macaques[38], mice[50] and zebrafish[19,38,51]. The discovery that PKD2L1 is a marker for CSF-cNs in the spinal cord led to the generation of stable transgenic lines of mice[9,40] and zebrafish[52] in which spinal CSF-cNs could be targeted for functional investigation using electrophysiology and optical methods[4,10,52–55].

In zebrafish and lamprey, ventral and dorsolateral CSF-cNs express distinct peptides and neuromodulators[12,19,42,56–60]. Immunohistochemistry **[G]** performed on diverse species (lampreys, turtles, fish, amphibians and macaques) and extended by recent transcriptomic analyses **[G]** in zebrafish[19] and mice[61] has confirmed these markers of CSF-cNs and uncovered numerous others, including a wide repertoire of neuropeptides and secreted proteins. In zebrafish, these include the urotensin related peptides (Urp1 and Urp2), somatostatin (Sst1.1), natriuretic peptide C (Nppc), prostate-associated microseminoprotein (Msmp), urocortin 3 (Ucn3), tachykinin 3 (Tac3) and secretogranin 2 (Scg2). In macaques, vasoactive intestinal peptide (VIP) has been identified as a marker[62], whereas in mice and rats the enzyme responsible for the synthesis of for trace amines (aromatic-L-amino-acid decarboxylase (AADC)) is expressed in CSF-cNs. CSF-cNs often produce monoamines: dopamine was reported to be produced by ventral CSF-cNs in lamprey[12,57–60], while tryptophan hydroxylase 2 (TPH2) enables the transient expression of serotonin in CSF-cNs in fish[42,56] and birds[63]. In zebrafish, CSF-cNs also express genes encoding structural proteins present in their apical extension (including Myosin-3b, Espin, and Cilia and flagella associated protein 57), as well as a great diversity of receptors for the neuropeptides listed above (such as Sst and Nppc), neurotransmitters and neuromodulators circulating in the CSF[4,19,50,64]. In rodents, CSF-cNs contain the P2X2 subunit of purinergic receptors[44]. These

observations indicate that CSF-cNs are equipped to act as homeostatic 'CSF taste receptor cells' that can detect and secrete active biomolecules to finely regulate their concentration in the CSF.

[H2] CSF-cN developmental origins

CSF-cNs are subdivided into a dorsolateral type (sometimes referred to as CSF-cNs' or KA') and a ventral type (CSF-cNs" or KA") in zebrafish[27], rat[65] and mouse[40]. In zebrafish embryos, dorsolateral and ventral CSF-cNs differentiate early at 10 and 10-15 hours post fertilization from the p3 and pMN progenitor domains, respectively [27,35,38,41]. Numerous studies[27–37] have uncovered a transient and differential role of Sonic Hedgehog and Delta–Notch signaling[31] in the specification of ventral CSF-cNs versus dorsolateral CSF-cNs. Consequently, the cascade of transcription factors involved in their differentiation differs between these two CSF-cN types. These cascades rely on the actions of the transcription factors OLIG2 (in dorsolateral CSF-cNs) or ASCL1 (in ventral ones)[31], as well as those of a combination of other transcription factors that include TAL1 and/or TAL2, GATA2 and/or GATA3[27] and CRB1[60]. These transcription factor cascades lead to the expression of Somatostatin 1.1, PKD2L1, PKD1L2 and glutamate decarboxylase 1 (GAD1) and/or GAD2 in dorsolateral CSF-cNs and URP1 and/ or UPR2, PKD2L1, PKD1L2 and GAD1 and/ or GAD2 in ventral ones. In mouse, CSF-cNs differentiate relatively late at embryonic day (E)14. As in zebrafish, they originate from different progenitor domains[66]: dorsolateral CSF-cNs originate from p2 and dorsal pOL progenitor domains and exhibit tonic firing, whereas ventral CSF-cNs originate from progenitors adjacent to the floor plate[40,66] and fire single spikes[31].

Although these cells originate from two different developmental domains, all CSF-cNs have exquisite and conserved electrophysiological characteristics across vertebrate species (see REF [27] for a synthetic comparison of CSF-cN properties across species). These characteristics include an extremely-high membrane resistance (>1 GΩ), which allowed — to our knowledge for the first time — the recording of single channel openings in vivo using whole-cell patch clamp[67]. Evidence for two physiological types of CSF-cNs have been described in fish, turtles and rodents, one of which exhibits tonic firing, while the other fires in a phasic manner[6,10,40,43,53,66–69]. These distinct firing patterns may be explained by differences in voltage-dependent

conductance[69], which is likely to arise from the actions of the two distinct transcription factor cascades that have been reported in the dorsolateral and ventral CSF-cNs[27–29,31,33,35,36,41,66,70].

Based on observations in *Platynereis dumerilii*, it has been suggested that CSF-cNs are closely related to ancestral neurons found in annelids. Neurons similar to CSF-cNs reside in the ventral nerve cord of *P. dumerilii*: these cells share the expression of GATA and TAL transcription factors and the transient synthesis of serotonin with CSF-cNs[71]. Similarly, the Reissner fibre is found in the central canal in both vertebrates and in the chordate amphioxus[21], further supporting the concept of an ancient origin for the axial sensory system. These observations suggest that neurons expressing GATA1, GATA2, GATA3 and TAL transcription factors in vertebrates may have evolved from CSF-cN–like sensory serotoninergic precursors involved in the regulation of ciliary locomotion in an ancestral bilaterian.

[H1] Polymodal interoceptive functions

[H2] Mechanosensory functions of CSF-cNs

As noted above, when Kolmer and Agduhr first observed CSF-cNs, they were struck by their peculiar morphology, which reminded them of hair cells, leading to the hypothesis that CSF-cNs could be mechanosensory cells in vivo[24] **(Fig. 2**; **Box 2)**. Later structural and morphological investigations further supported this hypothesis[14,21,72,73]. Recent studies in zebrafish and lamprey revisited this question and directly tested whether CSF-cNs have mechanosensory properties. In an open book preparation of the lamprey spinal cord, CSF-cNs responded to water jet application[5], suggesting that they might have a mechanosensory function and be able to detect changes of CSF flow. By taking advantage of the genetic and optical accessibility of larval zebrafish, it was shown that CSF-cNs detect active and passive changes in local spinal curvature in vivo[4]. Intriguingly, only the CSF-cNs situated on the concave side of the body axis responded to spinal curvature, showing that CSF-cNs respond to compression and not to stretch. Dorsolateral CSF-cNs are therefore activated by lateral compressions with cells on the left side of the zebrafish body responding to leftward bends of the body axis (with the reciprocal being

true for cells on the right side of the body)[4] (**Fig. 2a,b; Supplementary Video 4; Supplementary Video 5**), while ventral CSF-cNs respond to longitudinal compressions[74] (**Fig. 2c**). Direct mechanosensation was further demonstrated in in vitro experiments in which the CSF-cN membrane was mechanically-deformed, which modulates the open probability of the Pkd2l1 channel[67].

Due to the large conductance of the PKD2L1 channel (over hundreds of pS in mammalian slices[10] and in cultured cells[75]) and the high membrane resistance of CSF-cNs (> 1 GΩ in mouse[10,43], rat[76], zebrafish[7,67] and turtle[68,76]), the opening of a single PKD2L1 channel is sufficient to depolarize the cell and elicit action potential discharge[53,67]. In vitro, it has been shown that spontaneous channel opening probability also increases when applying pressure to the plasma membrane[67]. In the *pkd2l1* knock-out zebrafish mutant, spontaneous channel openings do not occur at baseline or upon pressure-application, suggesting that Pkd2l1 is necessary for the mechanosensory function of CSF-cNs[5,67]. In lamprey, acid sensing ion channels (ASICs) also contribute to mechanosensation in CSF-cNs, since the detection of fluid pressure-application is affected by an antagonist of ASIC3[5]. Whether CSF-cNs are also mechanosensory cells in mammals has not yet been reported. However, in acute brainstem slices from mice, hypo-osmotic shocks associated with CSF-cN swelling trigger a large increase in PKD2L1 channel open probability[10], suggesting that there may be direct mechanosensory function in mammalian CSF-cNs as well.

One question of interest is how CSF-cNs can detect spinal curvature in vivo[4]. At first, it was hypothesized that they respond to changes in the flow of CSF, because direct pressure application of artificial CSF elicits spiking[5]. Furthermore, CSF-cN spontaneous activity in embryos correlates with high CSF flow and reduces over time in the larval stage[67]. However, a response to CSF flow alone cannot explain the asymmetry of the response of CSF-cNs observed during a spinal bend[4], given that the profile of CSF flow is symmetrical: when the spinal cord bends in one direction, fundamental rules of fluid mechanics[77] indicate that the CSF flow will be maximal in the center and null on the walls of the central canal, but largely symmetrical within the central canal.

Recent evidence revealed that the Reissner fibre is essential to CSF-cN mechanosensory functions[7] (**Fig. 2; Supplemental Video 4; Supplementary Video 5**). Indeed, a recent preprint has suggested that CSF-cNs are functionally coupled to the Reissner fibre because their spontaneous activity relies on the intact fibre oscillating[78]. In larval zebrafish at rest, the Reissner fibre oscillates in the central canal and is under greatest tension towards its rostral side – possibly due to its interactions with ependymal beating cilia of caudal polarity[78,79]. Upon bending of the trunk, the Reissner fibre moves closer to the apical extension of CSF-cNs on the compressed (concave) side of the spinal cord and thereby can activate specifically CSF-cNs on this side[4,7]. The asymmetry in CSF-cN tuning to negative curvature therefore emerges from the fibre being under tension and moving closer to the side of compression. Accordingly, CSF-cN detection of spinal curvature is abolished in zebrafish *sspo* mutants that lack a Reissner fibre[6,7]. One can therefore postulate that the Reissner fibre can either directly interact with the apical extension of CSF-cNs — the 'physical contact' hypothesis[7] — or can enhance the flow perceived by the CSF-cN apical extension — the 'increased flow gradient' hypothesis[7]. In support of the physical contact hypothesis, the length of microvilli in the CSF-cN apical extension scales with the amplitude of the CSF-cN sensory response in zebrafish[64]. This observation suggests that the apical extension operates as a mechanosensory antenna.

In line with these observations, the ablation of cilia specifically in CSF-cNs in mice was sufficient to phenocopy the defects in skilled-locomotion observed upon ablation of the entire cells, suggesting that the CSF-cN cilium may be necessary for sensorimotor integration during locomotion in rodents[54]. In contrast to zebrafish larvae, in which loss of Pkd2l1 reduced kinematics during escape responses **[G]** [4], mice lacking PKD2L1 did not show motor defects[54]. However, before concluding that the effects of PKD2L1 are dispensable in mice, we should note that the effects observed in the mutant fish were subtle and the comparison had to be made across numerous siblings and clutches[4]. Investigations in mice have so far investigated the behavioral correlates of the PKD2L1 mutation on only a smaller number of animals. Furthermore, there are currently no reports demonstrating the mechanosensory function of CSF-cNs in mice and how it could be affected in PKD2L1 knock out animals. The specific role of the CSF-cN kinocilium and the nature of the interactions between the CSF-cN apical extension and the Reissner fibre will therefore require further mechanistic investigations in fish and mice.

[H2] Chemosensory functions of CSF-cNs

CSF-cNs are in an ideal location to detect chemical cues and changes in the composition of the CSF. The fact that PKD2L1 is both a marker of sour taste cells and of CSF-cNs led to the initial hypothesis that CSF-cNs were also chemosensory[9]. The ability of CSF-cNs to sense pH variations was proposed when an increase in spontaneous CSF-cN firing was observed in response to external pH acidification[9]. Since then, CSF-cNs have been shown to elicit action potential discharge in response to acidification and alkalinization of the artificial CSF in vitro (in recordings performed in mouse slices and in an open book preparation of the lamprey spinal cord[5,10], see **Fig. 3a**), through the opening of distinct cationic channels: ASICs for acidification and PKD2L1 channels for alkalinization in lamprey[5], mouse[10,53,80] and zebrafish[81] CSF-cNs. The location of PKD2L1 in the apical extension of macaque, mouse and fish CSF-cNs[38,67] suggest that these cells detect variations of pH in the CSF itself, although the expression of ASIC channels and their distribution within CSF-cN apical extension is unknown. Evidence for responses to acetylcholine[13,43] and ATP[13,44] in CSF-cNs have also been reported and the contribution of these neurons to the detection of neuromodulators and neurotransmitters released in the CSF should therefore be further investigated.

Although the first evidence for CSF-cN interoceptive functions came from observations of their responses to changes of pH and osmolarity, the corresponding physiological relevance is not yet fully understood. In contrast, recent evidence indicates that CSF-cNs can detect other chemical cues in the CSF such as metabolites produced by pathogenic bacteria and cells infected by viruses[19,82] (**Fig. 3b**). Such chemosensation has been observed in larval zebrafish upon invasion of the CSF by pathogenic bacteria, such as *Streptococcus pneumoniae* or *Listeria monocytogenes*. When either of these live bacterial species were directly injected in the brain ventricles, CSF-cN sparse activity was silenced and CSF-cNs instead exhibited exceptionally long and large calcium transients[19] (**Supplementary Video 6**). Accordingly, transcriptome analysis revealed that CSF-cNs specifically express multiple orphan bitter taste receptors **[G]** which may explain their in vitro responses to bacterial supernatant, bacterial metabolites and the supernatant of cells infected by neurotropic viruses[19] (**Supplementary Video 7**). These

observations indicate that CSF-cNs may ‘taste’ bacterial products in the CSF and respond by generating massive calcium transients during an infection.

[H2] Other putative sensory modalities of CSF-cNs

PKD2L1 is a nonselective cation channel that can be regulated by voltage, temperature, osmolarity, protons and calcium[83–87]. The PKD2L1 channel is inhibited by heat[87], which may explain the silencing of sparse activity in CSF-cNs during an infection associated with behavioral fever[88]. In addition, the opsin Valopa[89] has been identified in the zebrafish CSF-cN transcriptome, suggesting that CSF-cN activity may be light-modulated[19]. Similar findings on CSF-cNs from the paraventricular organ in quail showed that expression of opsin-5 and photosensitivity underlies the seasonal reproductive response[90]. Gating of the PKD2L1 channel is also linked to the membrane potential and impacts the response of CSF-cNs to changes in temperature and osmolarity[91].

[H1] CSF-cN downstream signalling

[H2] Neurosecretion

Although the process of secretion of biomolecules from particular cell types into the CSF is challenging to dynamically measure in vivo, multiple lines of evidence support the suggestion that CSF-cNs constitute an important neurosecretory system. The careful examination of the ultrastructure of CSF-cNs across species revealed the presence, in the axon terminals, of clear vesicles – likely containing GABA. In the apical extension, on the other hand, dense core secretory vesicles **[G]** — likely containing peptides or monoamines — were observed [6,21,42]. In numerous vertebrate species, CSF-cNs have been shown to express diverse peptides and monoamines. Sst was identified in lamprey, salmon and zebrafish CSF-cNs[2,42,92–97] and, as mentioned above, peptides of the Urotensin-2 family have been described in CSF-cNs of several fish species[15,93,94,98–100]. The dichotomy in the expression of Sst and urotensin-related peptides in the dorsolateral CSF-cNs and ventral CSF-cNs populations (see above), originally noticed in the

salmon[94] and confirmed in zebrafish[2,42, 92-93,95-96], has yet to be investigated in other species. In terms of monoamine localization, ventral CSF-cNs transiently synthesize serotonin in the zebrafish embryo[42] and dopamine throughout life in lamprey[59]. By contrast, mammalian CSF-cNs express the enzyme AADC[101] without producing serotonin or dopamine[102], suggesting that, under normal physiological conditions, these cells may produce trace amines[103–105]. Mammalian CSF-cNs, however, may produce monoamines after spinal cord injury[106–108]. The recently analyzed transcriptome of CSF-cNs in 3 day-old zebrafish larvae confirmed the expression of Sst1.1, Urp1 and Urp2, and revealed over 12 peptides highly enriched in these cells together with enzymes for the synthesis of serotonin and trace amines[19]. CSF-cNs express distinct peptides in mammals, such as the opioid peptide methionine-enkephalin-arginine-glycine-leucine in rat[109] and VIP in rat, cat and macaque[62]. In addition to the local modulation of spinal circuits (see below), the localization of dense core secretory vesicles underneath the apical extension suggest that CSF-cNs could release compounds that act at long distances by entering the CSF circulation at the level of the central canal[14] and in the subarachnoidal space[110,111].

Based on the work performed using optogenetic-mediated connectivity mapping (see below), we can infer that single spikes in CSF-cNs are sufficient to release clear vesicles containing GABA onto their targets within motor circuits in the spinal cord and hindbrain. By contrast, the pattern of activity necessary for release of dense core vesicles containing peptides, secreted proteins or monoamines is not known, but might include tonic firing as seen in other neurosecretory structures in the brain[112]. Although secretion has not been yet monitored in vivo, we can speculate that dense core vesicles could be released from the apical extension into the central canal. However, we cannot exclude the possibility that CSF-cNs could also release peptides on their basal side to reach receptors in the floor plate or notochord. Future studies will investigate which pattern of activity in CSF-cNs enables the secretion of dense core vesicles and whether they fuse with the membrane at the apical or basal pole in CSF-cNs.

[H2] Connectivity in hindbrain and spinal cord

Initial observations in sagittal sections of the spinal cord in rats and lamprey revealed that CSF-cN axons project in the basal lamina of the spinal cord[59,110,113,114] along the anteroposterior axis[44].

Mosaic labeling of CSF-cNs in larval zebrafish revealed that their axons ascend ipsilaterally in the ventral spinal cord, typically projecting over 100 - 300 µm (which corresponds to 2 - 6 segments in zebrafish larvae)[42,52]. While all CSF-cNs innervate the spinal cord, only the most rostral cells project to the hindbrain[2,42,52,55]. An indication that CSF-cNs project onto spinal central pattern generators **[G]** originated from experiments in larval zebrafish in which chemo-optogenetic activation of CSF-cNs led to a series of low frequency oscillations of the tail[2]. Later experiments revealed that optically-driven spiking of CSF-cNs can modulate the initiation and termination of slow locomotion as a function of the locomotor state[52]. Such modulation relies on release of GABA[52] and somatostatin[17].

Optogenetic-assisted connectivity mapping in zebrafish revealed that CSF-cN targets in the spinal cord consist of premotor excitatory interneurons[52], sensory interneurons and motor neurons[74], but not inhibitory interneurons (glycinergic or GABAergic[74]) (**Fig. 4**). CSF-cNs synapse in particular onto premotor excitatory V0-v interneurons involved in slow motion[52] and preliminary evidence indicated that they may also synapse on V2a spinal interneurons (C.W., unpublished observations). CSF-cNs powerfully inhibit specific motor neurons involved in fast swimming and postural control (primary motor neurons known as CaP neurons[74,115]. Immunohistochemical labeling of Sst and dopamine in lamprey also indicated that dorsolateral Sst-positive CSF-cNs synapse onto sensory 'edge cells' (mechanoreceptors that detect stretch of the spinal cord), while ventral dopamine-positive CSF-cNs synapse onto interneurons from the lateral column and local monoaminergic neurons[59]. Similarly, in mice, CSF-cNs have been shown to project onto other CSF-cNs[54,55], onto motor neurons of axial muscles in the medial motor column and onto glutamatergic premotor interneurons such as V0-c and V2a interneurons[54,55].

Overall, CSF-cNs in mice, like those in fish, project onto motor neurons and premotor excitatory neurons[54,55]. In addition, connections among CSF-cNs suggest that they form a highly-interconnected network of sensory cells – similar to those found between other inhibitory sensory neurons in lamprey and zebrafish [116,117]. Like other mechanosensory neurons, such as Rohon-Beard neurons[118,119], CSF-cNs project onto giant sensory interneurons (referred to CoPA neurons in zebrafish[120], and giant interneurons in lamprey[121]) that are thought to carry

information relative to corollary discharge **[G]** back to the brainstem[74]. CSF-cNs can therefore convey sensory feedback information to the brain both directly, from the most rostral CSF-cNs, and indirectly via these sensory interneurons.

In zebrafish, the most rostral CSF-cNs stabilize posture to avoid rolling (**Supplementary Video 8**) and synapse onto the soma and dendrite of occipital motor neurons in the caudal hindbrain (**Fig. 4**; **Supplementary Video 9**) [46]. Some of these occipital motor neurons innervate the axial muscle controlling the head position[46]. In the brainstem, reticulospinal neurons constitute command neurons determining the initiation and kinetics of movements by sending descending information to locomotor central pattern generators in the spinal cord[122]. CSF-cNs form axo-axonic synapses **[G]** onto reticulospinal neurons in the hindbrain as well as throughout the spinal cord[46] (**Supplementary Video 10**). CSF-cN presynaptic varicosities match postsynaptic densities in presynaptic boutons of the Mauthner cell and visual system homeobox 2 (VSX2)-positive medullary reticulospinal neurons that have an important role in shaping locomotion across vertebrates[123–128]. This apposition of presynaptic and inhibitory postsynaptic markers suggests that CSF-cNs could either provide presynaptic inhibition to the command neurons via GABA-B receptors or facilitate conduction of action potentials along the axons of reticulospinal neurons via GABA-A receptors (as recently demonstrated for GABAergic modulation of sensory feedback[129]). In mammals, CSF-cNs also densely project in the ventral fissure[44,55,130], where the axons of spinal interneurons (propriospinal neurons) project in the spinal cord. Further investigations should test whether CSF-cNs also synapse onto the axons of reticulospinal neurons and vestibulospinal neurons descending from the pons[131].

The investigation of CSF-cN connectivity map has not been exhaustive and we can expect many other neuronal targets to be discovered in the years to come. In particular, although a recent preprint indicates that CSF-cNs receive synaptic inputs from GABAergic and serotoninergic neurons in Rhesus monkeys[132], little is known in terms of their synaptic inputs other than from other CSF-cNs. Physiological recordings of CSF-cNs in the mouse revealed a modulation by GABA and glycine[10]. In addition, a few presynaptic terminals containing dense secretory vesicles contact the soma of CSF-cNs in mice, possibly reflecting a modulation by monoamines, glutamate, acetylcholine or neuropeptides[42,43,108,113,130,133].

[H1] Role in body axis and spine alignment

Recent evidence revealed that the sensory interface between the CSF and the spinal cord is crucial for morphogenesis (**Fig. 5**). The synchronous beating of cilia projecting from the ependyma of the ventricular walls can pattern the circulation of the CSF[134,135]. For example, in the central canal of the spinal cord of embryonic zebrafish, motile caudally-polarized cilia in the ventral wall generate a bidirectional CSF flow[6,67,136]. Defects in these motile cilia have been associated with defects in the alignment of the body axis of the zebrafish embryo[16,137] and in the axis of the spine in juvenile and adult fish[138]. These observations suggested that shaping the body axis of embryos and the spine morphogenesis in juvenile zebrafish may share similar mechanisms. One can postulate that a system to internally detect CSF flow and spinal curvature may provide information that is used to develop and maintain the straightness of the body axis at any stage. Researchers have therefore investigated whether CSF-cNs and the Reissner fibre could contribute to morphogenesis in zebrafish. Accordingly, *sspo* mutant embryos in which there is no Reissner fibre grow with a ventral curled-down body axis, despite exhibiting intact motile cilia and CSF flow in the central canal[6]. Similar curvature of the body axis is exhibited by mutant embryos with defective motile cilia, in which the lumen of the central canal does not open[136], the CSF does not flow in the central canal and the Reissner fibre does not form[6]. These converging lines of evidence indicate that the Reissner fibre plays a critical role in body axis straightening during embryogenesis.

Concomitant with these discoveries[6], it was revealed that the most downregulated genes in mutant embryos with ciliary defects encoded the urotensin-related peptides (Urp1 and Urp2), which are homologous to the vasoconstrictive human peptide urotensin-2[16]. Urp1 and Urp2 are expressed in the spinal cord in ventral CSF-cNs[15]. During embryogenesis, an upregulation of these peptides in the spinal cord is under the control of the Reissner fibre[139]: that is, if the Reissner fibre does not form during embryogenesis[140,16], *urp2* is downregulated and the body axis curls down. In these mutants, the overexpression of *urp1* or *urp2* is sufficient to rescue body axis curvature[16,140]. Remarkably, a straight body axis can be achieved in *sspo* mutant larvae by the sole expression of *urp2* in CSF-cNs[139]. However, subsequent genetic studies relying on

double *urp1,urp2* knock-outs found that these peptides were not mandatory for the straightening of the body axis[16,18,20,139], indicating that other molecular pathways under the control of the Reissner fibre may compensate for their loss during embryogenesis.

In contrast to these findings on body axis alignment during embryogenesis, there is evidence that Urp1 and/or Urp2 signaling becomes essential for spine alignment at the larval, juvenile and adult stages in zebrafish[18,20]. Loss of Urp1 and Urp2 led to spinal curvature at the larval stages[20] that subsequently developed in adults into planar curves in the caudal spine without structural deformations or vertebral patterning defects[18,20]. Similar spinal defects were observed in the mutant for the receptor of Urp1 and Urp2, encoded by *uts2r3*[20], suggesting that Urp1 and Urp2 released in the CSF at the level of the central canal can act at long distance on their targets in the dorsal musculature expressing the Uts2r3 receptor[16,20]. The action of Urp1 and/or Urp2 on the Uts2r3 receptor relies at the embryonic stage on an active mechanism of muscular contraction involving myosin-2[20].

In juvenile and adult zebrafish, evidence indicates that the sensory system formed by CSF-cNs and the Reissner fibre continues to shape spine morphology through Urp1 and/or Urp2 signaling. Urp1 and Urp2 are expressed by CSF-cNs until adulthood[15] and their expression is dysregulated in diverse scoliotic mutants[139,141,142]. *pkd2l1* mutants in which the CSF-cN mechanosensory function is reduced exhibit hyperkyphosis **[G]** in late adulthood with excessive ventral curvature at the level of the rostral spine[67], indicating a possible role of this pathway in deformations occurring with aging. Mutations affecting the maintenance of the Reissner fibre at the juvenile stages lead to drastic 3D torsion of the spine[79,139,141], mimicking the hallmark of idiopathic scoliosis **[G]** in humans.

Adrenergic signaling plays a very interesting role in the alignment of the body axis: catecholamines upregulate *urp1* and *urp2* expression in CSF-cNs and can rescue the curled-down phenotype exhibited when cilia are defective[16], or when the Reissner fibre does not form[139,140]. These observations indicate that the Reissner fibre is required during embryogenesis to enhance the expression of Urp peptides in the spinal cord, possibly via adrenergic signaling. Through this pathway, active dorsal contraction straightens the body axis and prevents the embryo from

curling down. The mechanisms by which the Reissner fibre and adrenergic signaling triggers the upregulation of *urp1* and/or *urp2* expression in the spinal cord are, however, not yet fully understood.

In humans, evidence of Urotensin-2–UTS2R3 signaling being altered in patients with adolescent idiopathic scoliosis[143,144] raises the question of whether the urotensin signaling pathway and CSF-cNs contribute to morphogenesis and body axis straightening in humans. In humans and great apes, the *sspo* pseudogene *SSPOP* has probably lost the ability to encode the complete SCO-spondin protein[145], indicating that the Reissner fiber itself may have been lost with the evolution of bipedalism about 11 million years ago. Future studies will therefore have to address whether CSF-cNs and Urotensin-2 might nonetheless contribute to posture and spine morphogenesis in humans. Nevertheless, the evidence that, in zebrafish, the expression and secretion of peptides of the urotensin-2 family in CSF-cNs enables the tonic activation of skeletal muscle cells in order to change posture and morphogenesis at the embryo, juvenile and adult state highlights the potential for CSF-cNs to act on distant targets via the CSF.

[H1] Role in locomotion and posture

As described above, CSF-cNs are located in the ventral spinal cord in close vicinity to motor neurons and locomotor central pattern generators, suggesting that these cells could actively adjust posture and locomotion. Optogenetic excitation of zebrafish CSF-cNs in vivo can trigger slow forward locomotion[2], revealing that CSF-cNs modulate the locomotor central pattern generators that drive forward locomotion (**Fig. 4**). Further investigations relying on recordings of motor patterns in paralyzed zebrafish (a preparation referred to as fictive locomotion) showed that CSF-cNs can impact spontaneous locomotion differentially depending on whether the animal is at rest or swimming[52]. Subsequent high-throughput analysis of kinematics during fast locomotion in freely-swimming animals revealed that CSF-cNs enhance the speed and power of fast locomotion of acousto-vestibular escape responses [4,46,74]. As mentioned above, dorsolateral CSF-cNs recruited by lateral bends and expressing Sst1.1 project onto excitatory premotor

interneurons (V0-v interneurons) in the spinal cord[42]. The *sst1.1* null mutant spontaneously swims for a longer duration than control animals[17], indicating that Sst contributes to the inhibition of spontaneous locomotion, possibly via this projection[52]. By contrast, ventral CSF-cNs recruited by longitudinal bending express Urp1 and Urp2 and project onto primary motor neurons in the spinal cord (CaP primary motor neurons)[74] and brainstem (occipital motor neurons)[46].

CSF-cNs also contribute to active postural control during fast locomotion[46,74] (**Fig. 4; Supplementary Video 8**). CSF-cNs in the most rostral part of the spinal cord stabilize larval zebrafish to avoid rolling after the initial large tail bend displayed at the start of an escape response (the so-called 'C-bend') and the counter-bends that occur during acousto-vestibular escapes[46] (**Supplementary Video 8**). This observation, combined with anatomical investigations, suggests that the rostral most CSF-cNs sense the initial head displacement that occurs at the beginning of a startle response[146], and feedback this angular information to occipital motor neurons and spinal motor neurons in order to maintain stability [46]. Because primary motor neurons in the occipital column and spinal cord project only ventrally and ipsilaterally onto axial skeletal muscles, their spiking leads to a ventral torque[147]. Consequently, when CSF-cN feedback is silenced by the ablation of CSF-cNs [46] or blockade of their secretory functions[74], larval zebrafish performing an escape response more often roll during the oscillation that follows the initial C-bend.

Recent work investigated the motor contribution of CSF-cNs to locomotor behaviour in mice[54,55]. The genetic ablation of CSF-cNs resulted in a reduced performance of skilled movements in the horizontal ladder and balanced beam tests[54] and a reduction of speed during treadmill locomotion[55]. These results indicate that the sensorimotor integrative function of CSF-cNs may be largely conserved between zebrafish and mice and point to CSF-cNs as an intraspinal sensory system that can also modulate motor output to fine tune locomotion in limbed animals. In terms of evolution, these results raise the fascinating question of how an axial sensory system, while apparently conserved throughout vertebrates to optimize speed and balance during skilled locomotion, has evolved to accommodate a different array of motor strategies and behavioral needs. Because this axial sensory system is directional, with axons

projecting only ipsilaterally and ascending[2,42,52,55], it may have evolved along with the rostrocaudal wave of excitation that underlies undulatory locomotion in aquatic swimming species (lamprey, xenopus, salamander and fish) and that appears to be conserved across segments including locomotor circuits in birds and mammals during development[148].

[H1] Role in host defense and innate immunity

Pathogenic bacteria penetrating the central nervous system invade the CSF during infections such as encephalitis or meningitis. Because the CSF-cN-mediated axial sensory system at the interface with the CSF is involved in postural control, one can postulate that it may be relevant for pathognosis of meningitis, and in particular for the stiffness of the neck and opisthotonos observed in meningitis patients. Accordingly, the infection of larval zebrafish with *S. pneumoniae* triggers sickness behavior with epileptic-like seizures and dorsal arching reminiscent of opisthotonos[19]. This suggests that the massive calcium transients in CSF-cNs observed late during the infection triggers the release of Urp1 and Urp2 and that the signaling of these peptides via the Uts2r3 receptor causes the body to curl up. Long and massive calcium transients occurring during infections (**Supplementary Video 6**) are indeed optimal for the release of dense core vesicles, which are typically situated far from the membrane compared to clear vesicles and therefore require more calcium influx to enable them to fuse with the membrane for their release [149].

The blockage of CSF-cN secretory release also reduces survival of the host during a *S. pneumoniae* infection, suggesting that secretory factors released by CSF-cNs such as neuropeptides, neuromodulators and secretory proteins contribute to host defense[19]. Additional evidence indicated that CSF-cNs participate in innate immunity via an upregulation of neurosecretion in the CSF[19] (**Fig. 3b**): transcriptome analysis revealed that, during the infection, CSF-cNs upregulate the expression of numerous cytokines and complement components, along with peptides previously shown to carry antimicrobial functions or to change tonic muscle activity and posture[19]. The mechanisms by which CSF-cN neurosecretion, possibly involving circulation in the CSF, can enhance survival of the host are, however, not completely understood.

In favor of an important role of CSF-cNs in the responses to diverse kind of infections of the CNS, *S. pneumoniae* is not the only pathogen activating CSF-cNs: *L. monocytogenes* and the supernatant of cells infected by the neurotropic Sindbis virus, can effectively trigger large calcium transients in CSF-cNs[19], suggesting that their role in defense against pathogens may be widespread.

[H1] Putative role as neural stem cells?

The soma of CSF-cNs is inserted in the region surrounding the central canal of the spinal cord, which is one of the adult neurogenic niches that can act as a source of endogenous repair across vertebrates[39]. As described above, electrophysiological recordings from CSF-cNs in vitro[10,40,53,68] and in vivo[67] revealed that they have a very high membrane resistance[10,67]. Such high membrane resistance is typical of immature neurons. Accordingly, spinal CSF-cNs exhibit numerous molecular markers of immature neurons such as low levels of NeuN, high levels of doublecortin (DCX)[19,103], the homeobox protein NKX6.1, ELAV-like protein 3 (ELAVL3, also known as HuC/D), ASCL1[66] and the polysialylated form of the neural cell adhesion molecule (PSA-NCAM) [44,47,65,68,69,76]. Based on these expression patterns, we can postulate that such immaturity conserved at the adult stage may confer CSF-cNs with a high degree of structural plasticity[150] and the ability to respond and regenerate after lesions. Indeed, mouse PKD2L1-positive CSF-cN neurospheres formed in vitro express neural stem cell markers, show proliferation and have the ability to differentiate into neurons, astrocytes, and oligodendrocytes[151]. Recent investigations of the stem cell potential of CSF-cNs in vivo confirmed that spinal cord injury or injection of neurotrophic factors (basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF)) into the lateral ventricle resulted in the activation and proliferation of CSF-cNs[152]. A previous study reported monoamine synthesis in CSF-cNs found to express aromatic L-amino acid decarboxylase (AADC) in response to injury [103,106,108]. Finally, indications of recovery post spinal cord injury uncovered an exceptional resilience of CSF-cNs in rodents that appear highly prioritized in lumbar spinal cord of mice that recovered walking with electrical stimulation[153]. CSF-cNs are dense in the adult spinal cord of primates[38,62,132]. Altogether, these findings highlight the neural stem cell potential of CSF-cNs that could contribute to the spinal response to an injury and subsequent repair.

[H1] Conclusions

As we have shown, central sensory neurons in contact with the CSF in the spinal cord can carry multiple physiological functions (**Fig. 1**): via their mechanosensitivity, CSF-cNs contribute to locomotion, postural control and morphogenesis throughout life. Via their chemosensitivity, CSF-cNs can respond to changes of the composition of the CSF, particularly when physiological pH and osmolarity vary or upon infection of the central nervous system. These central sensory neurons therefore resemble DRG neurons in the periphery that are also polymodal[154] (mechanosensory and/or chemosensory and/or thermosensory) and are involved in proprioception, pain and innate immunity upon bacterial infections following a skin cut[155]. The peculiarity of CSF-cNs, compared to DRG neurons, lies in their GABAergic phenotype, their ancestral origins and their ability to provide an interface between the nervous system and the complex CSF. By detecting and releasing compounds in the CSF, CSF-cNs can regulate its composition and distribute neuropeptides, neuromodulators or other bioactive molecules that can act over long distance outside of the central nervous system, reaching targets like skeletal muscles.

While most of the in vivo functional investigations we reported here were performed in zebrafish, investigations in other species, particularly in mice, show a remarkable level of conservation among vertebrate species. Future studies will inquire whether CSF-cNs are mechanosensory in mice, and how they rely on their cilia to do so. One can wonder how such an axial sensory system, which is apparently conserved across vertebrates in its capacity to enhance speed and stability during challenging locomotion, has evolved to provide meaningful sensory feedback for different motor strategies, including swimming and walking. As noted above, it is possible that it may have emerged concomitantly with the rostrocaudal wave of excitation enabling propulsion in aquatic swimming species, which show highly similar organization in terms of motor circuits[122,156]. Remarkably, the early stages of development in walking species, such as the chick embryo and the neonatal mouse, also show rostrocaudal wave of excitation corresponding to the execution of the first movements[148,157,158]. Scratching reflexes in adult cats also show excitatory traveling waves along the rostrocaudal axis of the spinal cord[159] and

rostrocaudal propagation of inhibition has been suggested in adult mice[160]. We can therefore speculate that ancient motifs of undulatory locomotion in aquatic species, including the excitatory rostal–caudal wave in segments on which locomotor central pattern generators are located have been coupled to directional interoceptive feedback and therefore maintained together throughout evolution in vertebrate species.

How CSF-cNs integrate multiple kinds of cues and release the content of dense core vesicles remains a mystery. Furthermore, recent reports in mice uncovered the potential of CSF-cNs to act as neural stem cells in the adult spinal cord after injury, suggesting that these cells may be valuable targets for endogenous repair in the spinal cord.

Future investigations will be required to determine which elements of this sensory system are conserved in humans. Physiological experiments in ex-vivo preparations of the mouse spinal cord, together with clearing methods in the macaque and human spinal cord should shed some light onto the cellular components of this sensory system and the mechanisms at play for interoception in the human spinal cord.

Furthermore, future investigations will be required to uncover the physiological roles of CSF-cNs in the brain ventricles, determining whether they, together with those in the spinal cord, form a wide signaling and neurosecretory network linking the brain to effectors in the rest of the body.

References

1. Rasmussen, M. K., Mestre, H. & Nedergaard, M. Fluid transport in the brain. *Physiol. Rev.* **102**, 1025–1151 (2022).
2. Wyart, C. *et al.* Optogenetic dissection of a behavioural module in the vertebrate spinal cord. *Nature* **461**, 407–410 (2009).

3. Orts-Del'Immagine, A. & Wyart, C. Cerebrospinal-fluid-contacting neurons. *Curr. Biol.* **27**, R1198–R1200 (2017).
4. Böhm, U. L. *et al.* CSF-contacting neurons regulate locomotion by relaying mechanical stimuli to spinal circuits. *Nat. Commun.* **7**, 10866 (2016).
5. Jalalvand, E., Robertson, B., Wallén, P. & Grillner, S. Ciliated neurons lining the central canal sense both fluid movement and pH through ASIC3. *Nat. Commun.* **7**, 10002 (2016).
6. Cantaut-Belarif, Y., Sternberg, J. R., Thouvenin, O., Wyart, C. & Bardet, P.-L. The Reissner Fiber in the Cerebrospinal Fluid Controls Morphogenesis of the Body Axis. *Curr. Biol.* **28**, 2479-2486.e4 (2018).
7. Orts-Del'Immagine, A. *et al.* Sensory Neurons Contacting the Cerebrospinal Fluid Require the Reissner Fiber to Detect Spinal Curvature In Vivo. *Curr. Biol.* **30**, 827-839.e4 (2020).
8. Eccles, J. C. & Sherrington, C. S. Reflex summation in the ipsilateral spinal flexion reflex. *J. Physiol.* **69**, 1–28 (1930).
9. Huang, A. L. *et al.* The cells and logic for mammalian sour taste detection. *Nature* **442**, 934–938 (2006).
10. Orts-Del'Immagine, A. *et al.* Properties of subependymal cerebrospinal fluid contacting neurones in the dorsal vagal complex of the mouse brainstem. *J. Physiol.* **590**, 3719–3741 (2012).
11. Jalalvand, E., Robertson, B., Tostivint, H., Wallén, P. & Grillner, S. The Spinal Cord Has an Intrinsic System for the Control of pH. *Curr. Biol.* **26**, 1346–1351 (2016).
12. Jalalvand, E. *et al.* ExSTED microscopy reveals contrasting functions of dopamine and somatostatin CSF-c neurons along the lamprey central canal. *eLife* **11**, e73114 (2022).

13. Johnson, E. *et al.* Graded spikes differentially signal neurotransmitter input in cerebrospinal fluid contacting neurons of the mouse spinal cord. *iScience* **26**, 105914 (2023).
14. Vigh, B. & Vigh-Teichmann, I. Actual problems of the cerebrospinal fluid-contacting neurons. *Microsc. Res. Tech.* **41**, 57–83 (1998).
15. Quan, F. B. *et al.* Comparative Distribution and In Vitro Activities of the Urotensin II-Related Peptides URP1 and URP2 in Zebrafish: Evidence for Their Colocalization in Spinal Cerebrospinal Fluid-Contacting Neurons. *PLOS ONE* **10**, e0119290 (2015).
16. Zhang, X. *et al.* Cilia-driven cerebrospinal fluid flow directs expression of urotensin neuropeptides to straighten the vertebrate body axis. *Nat. Genet.* **50**, 1666–1673 (2018).
17. Quan, F. B. *et al.* Somatostatin 1.1 contributes to the innate exploration of zebrafish larva. *Sci. Rep.* **10**, 15235 (2020).
18. Bearce, E. A. *et al.* Urotensin II-related peptides, Urp1 and Urp2, control zebrafish spine morphology. *eLife* **11**, e83883 (2022).
19. Prendergast, A. E. *et al.* CSF-contacting neurons respond to Streptococcus pneumoniae and promote host survival during central nervous system infection. *Curr. Biol.* **33**, 940-956.e10 (2023).
20. Gaillard, A.-L. *et al.* Urp1 and Urp2 act redundantly to maintain spine shape in zebrafish larvae. *Dev. Biol.* **496**, 36–51 (2023).
21. Vigh-Teichmann, I. & Vigh, B. The system of cerebrospinal fluid-contacting neurons. *Arch. Histol. Jpn.* **46**, 427–468 (1983).
22. Vígh, B. *et al.* The system of cerebrospinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain. *Histol. Histopathol.* 607–628 (2004) doi:10.14670/HH-19.607.

23. Djenoune, L. & Wyart, C. Light on a sensory interface linking the cerebrospinal fluid to motor circuits in vertebrates. *J. Neurogenet.* **31**, 113–127 (2017).
24. Kolmer, W. Das 'Sagitallorgan' der Wirbeltiere. *Z Anat EntwGesch* 652–717 (1921).
25. Agduhr, E. Über ein zentrales Sinnesorgan (?) bei den Vertebraten. *Z. Für Anat. Entwicklungsgeschichte* **66**, 223–360 (1922).
26. Dale, N., Roberts, A., Otterson & Storm-Mathisen, J. The morphology and distribution of `Kolmer–Agduhr cells', a class of cerebrospinal-fluid-contacting neurons revealed in the frog embryo spinal cord by GABA immunocytochemistry. *Proc. R. Soc. Lond. B Biol. Sci.* **232**, 193–203 (1987).
27. Yang, L., Rastegar, S. & Strähle, U. Regulatory interactions specifying Kolmer-Agduhr interneurons. *Development* **137**, 2713–2722 (2010).
28. Zheng, P.-F., Xie, H.-B., Zhu, P.-P. & Zhao, C.-T. Distribution pattern of floor plate neurons in zebrafish. *Yi Chuan Hered.* **44**, 510–520 (2022).
29. Jacobs, C. T., Kejriwal, A., Kocha, K. M., Jin, K. Y. & Huang, P. Temporal cell fate determination in the spinal cord is mediated by the duration of Notch signalling. *Dev. Biol.* **489**, 1–13 (2022).
30. Cunningham, R. L. *et al.* Functional in vivo characterization of sox10 enhancers in neural crest and melanoma development. *Commun. Biol.* **4**, 695 (2021).
31. Yang, L., Wang, F. & Strähle, U. The Genetic Programs Specifying Kolmer–Agduhr Interneurons. *Front. Neurosci.* **14**, (2020).
32. Andrzejczuk, L. A. *et al.* Tal1, Gata2a, and Gata3 Have Distinct Functions in the Development of V2b and Cerebrospinal Fluid-Contacting KA Spinal Neurons. *Front. Neurosci.* **12**, 170 (2018).

33. Ryu, J.-H. *et al.* Generation of late-born neurons in the ventral spinal cord requires the coordination of retinoic acid and Notch signaling. *Neurosci. Lett.* **602**, 95–98 (2015).

34. Seredick, S., Hutchinson, S. A., Van Ryswyk, L., Talbot, J. C. & Eisen, J. S. Lhx3 and Lhx4 suppress Kolmer-Agduhr interneuron characteristics within zebrafish axial motoneurons. *Dev. Camb. Engl.* **141**, 3900–3909 (2014).

35. Huang, P., Xiong, F., Megason, S. G. & Schier, A. F. Attenuation of Notch and Hedgehog signaling is required for fate specification in the spinal cord. *PLoS Genet.* **8**, e1002762 (2012).

36. Yeo, S.-Y. & Chitnis, A. B. Jagged-mediated Notch signaling maintains proliferating neural progenitors and regulates cell diversity in the ventral spinal cord. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 5913–5918 (2007).

37. Binor, E. & Heathcote, R. D. Development of GABA-immunoreactive neuron patterning in the spinal cord. *J. Comp. Neurol.* **438**, 1–11 (2001).

38. Djenoune, L. *et al.* Investigation of spinal cerebrospinal fluid-contacting neurons expressing PKD2L1: evidence for a conserved system from fish to primates. *Front. Neuroanat.* **8**, 26 (2014).

39. Becker, C. G., Becker, T. & Hugnot, J.-P. The spinal ependymal zone as a source of endogenous repair cells across vertebrates. *Prog. Neurobiol.* **170**, 67–80 (2018).

40. Petracca, Y. L. *et al.* The late and dual origin of cerebrospinal fluid-contacting neurons in the mouse spinal cord. *Development* **143**, 880–891 (2016).

41. Shin, J., Poling, J., Park, H.-C. & Appel, B. Notch signaling regulates neural precursor allocation and binary neuronal fate decisions in zebrafish. *Development* **134**, 1911–1920 (2007).

42. Djenoune, L. *et al.* The dual developmental origin of spinal cerebrospinal fluid-contacting neurons gives rise to distinct functional subtypes. *Sci. Rep.* **7**, 719 (2017).

43. Corns, L. F. *et al.* Cholinergic Enhancement of Cell Proliferation in the Postnatal Neurogenic Niche of the Mammalian Spinal Cord. *Stem Cells Dayt. Ohio* **33**, 2864–2876 (2015).

44. Stoeckel, M.-E. *et al.* Cerebrospinal fluid-contacting neurons in the rat spinal cord, a gamma-aminobutyric acidergic system expressing the P2X2 subunit of purinergic receptors, PSA-NCAM, and GAP-43 immunoreactivities: light and electron microscopic study. *J. Comp. Neurol.* **457**, 159–174 (2003).

45. Barber, R. P., Vaughn, J. E. & Roberts, E. The cytoarchitecture of GABAergic neurons in rat spinal cord. *Brain Res.* **238**, 305–328 (1982).

46. Wu, M.-Y. *et al.* Spinal sensory neurons project onto the hindbrain to stabilize posture and enhance locomotor speed. *Curr. Biol. CB* **31**, 3315-3329.e5 (2021).

47. Orts-Del'Immagine, A. *et al.* Morphology, Distribution and Phenotype of Polycystin Kidney Disease 2-like 1-Positive Cerebrospinal Fluid Contacting Neurons in The Brainstem of Adult Mice. *PLoS ONE* **9**, e87748 (2014).

48. Alfaro-Cervello, C., Soriano-Navarro, M., Mirzadeh, Z., Alvarez-Buylla, A. & Garcia-Verdugo, J. M. Biciliated ependymal cell proliferation contributes to spinal cord growth. *J. Comp. Neurol.* **520**, 3528–3552 (2012).

49. Muñoz, R. I. *et al.* The subcommissural organ and the Reissner fiber: old friends revisited. *Cell Tissue Res.* **375**, 507–529 (2019).

50. Rosenberg, A. B. *et al.* Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding. *Science* **360**, 176–182 (2018).

51. England, S. J., Campbell, P. C., Banerjee, S., Swanson, A. J. & Lewis, K. E. Identification and Expression Analysis of the Complete Family of Zebrafish pkd Genes. *Front. Cell Dev. Biol.* **5**, (2017).

52. Fidelin, K. *et al.* State-Dependent Modulation of Locomotion by GABAergic Spinal Sensory Neurons. *Curr. Biol.* **25**, 3035–3047 (2015).

53. Orts-Del'Immagine, A. *et al.* A single polycystic kidney disease 2-like 1 channel opening acts as a spike generator in cerebrospinal fluid-contacting neurons of adult mouse brainstem. *Neuropharmacology* **101**, 549–565 (2016).

54. Gerstmann, K. *et al.* The role of intraspinal sensory neurons in the control of quadrupedal locomotion. *Curr. Biol. CB* **32**, 2442-2453.e4 (2022).

55. Nakamura, Y. *et al.* Cerebrospinal fluid-contacting neuron tracing reveals structural and functional connectivity for locomotion in the mouse spinal cord. *eLife* **12**, e83108 (2023).

56. Montgomery, J. E., Wiggin, T. D., Rivera-Perez, L. M., Lillesaar, C. & Masino, M. A. Intraspinal serotonergic neurons consist of two, temporally distinct populations in developing zebrafish. *Dev. Neurobiol.* **76**, 673–687 (2016).

57. Rodicio, M. C., Villar-Cerviño, V., Barreiro-Iglesias, A. & Anadón, R. Colocalization of dopamine and GABA in spinal cord neurones in the sea lamprey. *Brain Res. Bull.* **76**, 45–49 (2008).

58. Barreiro-Iglesias, A., Villar-Cerviño, V., Anadón, R. & Rodicio, M. C. Descending brain-spinal cord projections in a primitive vertebrate, the lamprey: cerebrospinal fluid-contacting and dopaminergic neurons. *J. Comp. Neurol.* **511**, 711–723 (2008).

59. Schotland, J. L., Shupliakov, O., Grillner, S. & Brodin, L. Synaptic and nonsynaptic monoaminergic neuron systems in the lamprey spinal cord. *J. Comp. Neurol.* **372**, 229–244 (1996).

60. McPherson, D. R. & Kemnitz, C. P. Modulation of lamprey fictive swimming and motoneuron physiology by dopamine, and its immunocytochemical localization in the spinal cord. *Neurosci. Lett.* **166**, 23–26 (1994).

61. Russ, D. E. *et al.* A harmonized atlas of mouse spinal cord cell types and their spatial organization. *Nat. Commun.* **12**, 5722 (2021).

62. Lamotte, C. C. Vasoactive intestinal polypeptide cerebrospinal fluid-contacting neurons of the monkey and cat spinal central canal. *J. Comp. Neurol.* **258**, 527–541 (1987).

63. Hirunagi, K., Hasegawa, M., Vigh, B. & Vigh-Teichmann, I. Immunocytochemical demonstration of serotonin-immunoreactive cerebrospinal fluid-contacting neurons in the paraventricular organ of pigeons and domestic chickens. *Prog. Brain Res.* **91**, 327–330 (1992).

64. Desban, L. *et al.* Regulation of the apical extension morphogenesis tunes the mechanosensory response of microvilliated neurons. *PLOS Biol.* **17**, e3000235 (2019).

65. Kútna, V., Ševc, J., Gombalová, Z., Matiašová, A. & Daxnerová, Z. Enigmatic cerebrospinal fluid-contacting neurons arise even after the termination of neurogenesis in the rat spinal cord during embryonic development and retain their immature-like characteristics until adulthood. *Acta Histochem.* **116**, 278–285 (2014).

66. Bella, D. J. D. *et al.* Ascl1 Balances Neuronal versus Ependymal Fate in the Spinal Cord Central Canal. *Cell Rep.* **28**, 2264-2274.e3 (2019).

67. Sternberg, J. R. *et al.* Pkd2l1 is required for mechanoception in cerebrospinal fluid-contacting neurons and maintenance of spine curvature. *Nat. Commun.* **9**, 3804 (2018).

68. Reali, C., Fernández, A., Radmilovich, M., Trujillo-Cenóz, O. & Russo, R. E. GABAergic signalling in a neurogenic niche of the turtle spinal cord. *J. Physiol.* **589**, 5633–5647 (2011).

69. Marichal, N., Garcia, G., Radmilovich, M., Trujillo-Cenoz, O. & Russo, R. E. Enigmatic Central Canal Contacting Cells: Immature Neurons in 'Standby Mode'? *J. Neurosci.* **29**, 10010–10024 (2009).

70. Park, H.-C., Shin, J. & Appel, B. Spatial and temporal regulation of ventral spinal cord precursor specification by Hedgehog signaling. *Dev. Camb. Engl.* **131**, 5959–5969 (2004).

71. Vergara, H. M. *et al.* Whole-organism cellular gene-expression atlas reveals conserved cell types in the ventral nerve cord of Platynereis dumerilii. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 5878–5885 (2017).

72. Vigh-Teichmann, I. & Vigh, B. The cerebrospinal fluid-contacting neuron: a peculiar cell type of the central nervous system. Immunocytochemical aspects. *Arch. Histol. Cytol.* **52 Suppl**, 195–207 (1989).

73. Vigh, B. & Vigh-Teichmann, I. Comparative ultrastructure of the cerebrospinal fluid-contacting neurons. *Int. Rev. Cytol.* **35**, 189–251 (1973).

74. Hubbard, J. M. *et al.* Intraspinal Sensory Neurons Provide Powerful Inhibition to Motor Circuits Ensuring Postural Control during Locomotion. *Curr. Biol.* **26**, 2841–2853 (2016).

75. Shimizu, T., Janssens, A., Voets, T. & Nilius, B. Regulation of the murine TRPP3 channel by voltage, pH, and changes in cell volume. *Pflüg. Arch. - Eur. J. Physiol.* **457**, 795–807 (2008).

76. Russo, R. E., Fernández, A., Reali, C., Radmilovich, M. & Trujillo-Cenóz, O. Functional and molecular clues reveal precursor-like cells and immature neurones in the turtle spinal cord. *J. Physiol.* **560**, 831–838 (2004).

77. *Fundamental Directions in Mathematical Fluid Mechanics*. (Birkhäuser Basel, 2000).

132. Bellegarda, C. *et al.* The Reissner fiber under tension in vivo shows dynamic interaction with ciliated cells contacting the cerebrospinal fluid. 2023.02.22.529498 Preprint at https://doi.org/10.1101/2023.02.22.529498 (2023).

79. Troutwine, B. R. *et al.* The Reissner Fiber Is Highly Dynamic In Vivo and Controls Morphogenesis of the Spine. *Curr. Biol.* **30**, 2353-2362.e3 (2020).

80. Bushman, J. D., Ye, W. & Liman, E. R. A proton current associated with sour taste: distribution and functional properties. *FASEB J.* **29**, 3014–3026 (2015).

81. Böhm, U. L. Physiological inputs to cerebrospinal fluid-contacting neurons. (Université Pierre et Marie Curie, 2016).

82. MacCain, W. & Tuomanen, E. Taste vs. pain: A sensory feast in bacterial meningitis. *Cell Host Microbe* **31**, 681–682 (2023).

83. Holzer, P. Acid-sensitive ion channels and receptors. *Handb. Exp. Pharmacol.* 283–332 (2009) doi:10.1007/978-3-540-79090-7_9.

84. Tian, P.-F., Sun, M.-M., Hu, X.-Y., Du, J. & He, W. TRPP2 ion channels: The roles in various subcellular locations. *Biochimie* **201**, 116–127 (2022).

85. Cantero, M. D. R. & Cantiello, H. F. Polycystin-2 (TRPP2): Ion channel properties and regulation. *Gene* **827**, 146313 (2022).

86. Su, Q. *et al.* Structural basis for Ca2+ activation of the heteromeric PKD1L3/PKD2L1 channel. *Nat. Commun.* **12**, 4871 (2021).

87. Higuchi, T., Shimizu, T., Fujii, T., Nilius, B. & Sakai, H. Gating modulation by heat of the polycystin transient receptor potential channel PKD2L1 (TRPP3). *Pflugers Arch.* **466**, 1933–1940 (2014).

88. Boltana, S. *et al.* Behavioral Fever Drives Epigenetic Modulation of the Immune Response in Fish. *Front. Immunol.* **9**, 1241 (2018).

89. Friedmann, D., Hoagland, A., Berlin, S. & Isacoff, E. Y. A spinal opsin controls early neural activity and drives a behavioral light response. *Curr. Biol. CB* **25**, 69–74 (2015).

90. Nakane, Y., Shimmura, T., Abe, H. & Yoshimura, T. Intrinsic photosensitivity of a deep brain photoreceptor. *Curr. Biol. CB* **24**, R596-597 (2014).

91. Ng, L. C. T., Vien, T. N., Yarov-Yarovoy, V. & DeCaen, P. G. Opening TRPP2 (PKD2L1) requires the transfer of gating charges. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 15540–15549 (2019).

92. Buchanan, J. T., Brodin, L., Hökfelt, T., Dongen, P. A. M. V. & Grillner, S. Survey of neuropeptide-like immunoreactivity in the lamprey spinal cord. *Brain Res.* **408**, 299–302 (1987).

93. Yulis, C. R. & Lederis, K. Occurrence of an anterior spinal, cerebrospinal fluid-contacting, urotensin II neuronal system in various fish species. *Gen. Comp. Endocrinol.* **70**, 301–311 (1988).

94. Yulis, C. R. & Lederis, K. Relationship between urotensin II- and somatostatin-immunoreactive spinal cord neurons of Catostomus commersoni and Oncorhynchus kisutch (Teleostei). *Cell Tissue Res.* **254**, 539–542 (1988).

95. Christenson, J., Alford, S., Grillner, S. & Hökfelt, T. Co-localized GABA and somatostatin use different ionic mechanisms to hyperpolarize target neurons in the lamprey spinal cord. *Neurosci. Lett.* **134**, 93–97 (1991).

96. López, J. M. *et al.* Distribution of somatostatin-like immunoreactivity in the brain of the caecilian Dermophis mexicanus (Amphibia: Gymnophiona): comparative aspects in amphibians. *J. Comp. Neurol.* **501**, 413–430 (2007).

97. Jalalvand, E., Robertson, B., Wallén, P., Hill, R. H. & Grillner, S. Laterally Projecting Cerebrospinal Fluid-Contacting Cells in the Lamprey Spinal Cord Are of Two Distinct Types. *J. Comp. Neurol.* **522**, Spc1–Spc1 (2014).

98. Yulis, C. R. & Lederis, K. The distribution of `extraurophyseal' urotensin I-immunoreactivity in the central nervous system of Catostomus commersoni after urophysectomy. *Neurosci. Lett.* **70**, 75–80 (1986).

99. Yulis, C. R. & Lederis, K. Extraurophyseal distribution of urotensin II immunoreactive neuronal perikarya and their processes. *Proc. Natl. Acad. Sci.* **83**, 7079–7083 (1986).

100. Yulis, C. R., Lederis, K., Wong, K.-L. & Fisher, A. W. F. Localization of urotensin I- and corticotropin-releasing factor-like immunoreactivity in the central nervous system of Catostomus commersoni. *Peptides* **7**, 79–86 (1986).

101. Jaeger, C. B. *et al.* Some Neurons of the Rat Central Nervous System Contain Aromatic-L-Amino-Acid Decarboxylase But Not Monoamines. *Science* **219**, 1233–1235 (1983).

102. Nagatsu, I., Sakai, M., Yoshida, M. & Nagatsu, T. Aromatic L-amino acid decarboxylase-immunoreactive neurons in and around the cerebrospinal fluid-contacting neurons of the central canal do not contain dopamine or serotonin in the mouse and rat spinal cord. *Brain Res.* **475**, 91–102 (1988).

103. Ren, L.-Q. *et al.* Heterogenic Distribution of Aromatic L-Amino Acid Decarboxylase Neurons in the Rat Spinal Cord. *Front. Integr. Neurosci.* **11**, 31 (2017).

104. Chatelin, S. *et al.* Neuronal promoter of human aromatic l-amino acid decarboxylase gene directs transgene expression to the adult floor plate and aminergic nuclei induced by the isthmus. *Mol. Brain Res.* **97**, 149–160 (2001).

105. Pierre, J., Mahouche, M., Suderevskaya, E. I., Repérant, J. & Ward, R. Immunocytochemical localization of dopamine and its synthetic enzymes in the central nervous system of the lamprey Lampetra fluviatilis. *J. Comp. Neurol.* **380**, 119–135 (1997).

106. Ren, L.-Q., Wienecke, J., Hultborn, H. & Zhang, M. Production of Dopamine by Aromatic l-Amino Acid Decarboxylase Cells after Spinal Cord Injury. *J. Neurotrauma* **33**, 1150–1160 (2016).

107. Azam, B., Wienecke, J., Jensen, D. B., Azam, A. & Zhang, M. Spinal Cord Hemisection Facilitates Aromatic L-Amino Acid Decarboxylase Cells to Produce Serotonin in the Subchronic but Not the Chronic Phase. *Neural Plast.* **2015**, 1–10 (2015).

108. Wienecke, J. *et al.* Spinal Cord Injury Enables Aromatic l-Amino Acid Decarboxylase Cells to Synthesize Monoamines. *J. Neurosci.* **34**, 11984–12000 (2014).

109. Shimosegawa, T. *et al.* An immunohistochemical study of methionine-enkephalin-Arg6-Gly7-Leu8-like immunoreactivity-containing liquor-contacting neurons (LCNs) in the rat spinal cord. *Brain Res.* **379**, 1–9 (1986).

110. Vigh, B., Vigh-Teichmann, I. & Aros, B. Special dendritic and axonal endings formed by the cerebrospinal fluid contacting neurons of the spinal cord. *Cell Tissue Res.* **183**, 541–552 (1977).

111. Megías, M., Álvarez-Otero, R. & Pombal, M. A. Calbindin and calretinin immunoreactivities identify different types of neurons in the adult lamprey spinal cord. *J. Comp. Neurol.* **455**, 72–85 (2002).

112. Morris, J. F. Neurosecretion (Regulated Exocytosis in Neuroendocrine Cells). in *Encyclopedia of Neuroscience* (ed. Squire, L. R.) 1007–1014 (Academic Press, 2009). doi:10.1016/B978-008045046-9.01189-X.

113. Vigh, B., Vigh-Teichmann, I., Silva, M. J. M. e & Pol, A. N. van den. Cerebrospinal fluid-contacting neurons of the central canal and terminal ventricle in various vertebrates. *Cell Tissue Res.* **231**, 615–621 (1983).

114. Baumgarten, H. G. Biogenic Monoamines in the Cyclostome and Lower Vertebrate Brain. *Prog. Histochem. Cytochem.* **4**, III–88 (1972).

115. Sternberg, J. R. *et al.* Optimization of a Neurotoxin to Investigate the Contribution of Excitatory Interneurons to Speed Modulation In Vivo. *Curr. Biol.* **26**, 2319–2328 (2016).

116. Picton, L. D. *et al.* Developmental switch in the function of inhibitory commissural V0d interneurons in zebrafish. *Curr. Biol. CB* **32**, 3515-3528.e4 (2022).

117. Di Prisco, G. V., Wallén, P. & Grillner, S. Synaptic effects of intraspinal stretch receptor neurons mediating movement-related feedback during locomotion. *Brain Res.* **530**, 161–166 (1990).

118. Knogler, L. D. & Drapeau, P. Sensory gating of an embryonic zebrafish interneuron during spontaneous motor behaviors. *Front. Neural Circuits* **8**, 121 (2014).

119. Liu, Y.-C. & Hale, M. E. Local Spinal Cord Circuits and Bilateral Mauthner Cell Activity Function Together to Drive Alternative Startle Behaviors. *Curr. Biol. CB* **27**, 697–704 (2017).

120. Hale, M. E., Ritter, D. A. & Fetcho, J. R. A confocal study of spinal interneurons in living larval zebrafish. *J. Comp. Neurol.* **437**, 1–16 (2001).

121. Homma, S. Conductance changes during bath application of $\upbeta$-alanine and taurine in giant interneurons of the isolated lamprey spinal cord. *Brain Res.* **173**, 287–293 (1979).

122. Kiehn, O. Decoding the organization of spinal circuits that control locomotion. *Nat. Rev. Neurosci.* **17**, 224–238 (2016).

123. Kimura, Y. *et al.* Hindbrain V2a Neurons in the Excitation of Spinal Locomotor Circuits during Zebrafish Swimming. *Curr. Biol.* **23**, 843–849 (2013).

124. Bouvier, J. *et al.* Descending Command Neurons in the Brainstem that Halt Locomotion. *Cell* **163**, 1191–1203 (2015).

125. Pujala, A. & Koyama, M. Chronology-based architecture of descending circuits that underlie the development of locomotor repertoire after birth. *eLife* **8**, e42135 (2019).

126. Cregg, J. M. *et al.* Brainstem neurons that command mammalian locomotor asymmetries. *Nat. Neurosci.* **23**, 730–740 (2020).

127. Schwenkgrub, J., Harrell, E. R., Bathellier, B. & Bouvier, J. Deep imaging in the brainstem reveals functional heterogeneity in V2a neurons controlling locomotion. *Sci. Adv.* **6**, eabc6309 (2020).

128. Usseglio, G., Gatier, E., Heuzé, A., Hérent, C. & Bouvier, J. Control of Orienting Movements and Locomotion by Projection-Defined Subsets of Brainstem V2a Neurons. *Curr. Biol.* **30**, 4665-4681.e6 (2020).

129. Hari, K. *et al.* GABA facilitates spike propagation through branch points of sensory axons in the spinal cord. *Nat. Neurosci.* **25**, 1288–1299 (2022).

130. Jurčić, N., Michelle, C., Trouslard, J., Wanaverbecq, N. & Kastner, A. Evidence for PKD2L1-positive neurons distant from the central canal in the ventromedial spinal cord and medulla of the adult mouse. *Eur. J. Neurosci.* **54**, 4781–4803 (2021).

131. Watson, C. & Harvey, A. R. Chapter 11 - Projections from the Brain to the Spinal Cord. in *The Spinal Cord* (eds. Watson, C., Paxinos, G. & Kayalioglu, G.) 168–179 (Academic Press, 2009). doi:10.1016/B978-0-12-374247-6.50015-8.

132. Kastner, A. & Wanaverbecq, N. In Rhesus monkeys, CSF-contacting neurons are the only neurons present in the medullo-spinal peri-ependymal zone. 2023.03.29.534787 Preprint at https://doi.org/10.1101/2023.03.29.534787 (2023).

133. Jurčić, N. *et al.* GABAB receptors modulate Ca2+ but not G protein-gated inwardly rectifying K+ channels in cerebrospinal-fluid contacting neurones of mouse brainstem. *J. Physiol.* **597**, 631–651 (2019).

134. Faubel, R., Westendorf, C., Bodenschatz, E. & Eichele, G. Cilia-based flow network in the brain ventricles. *Science* **353**, 176–178 (2016).

135. Olstad, E. W. *et al.* Ciliary Beating Compartmentalizes Cerebrospinal Fluid Flow in the Brain and Regulates Ventricular Development. *Curr. Biol.* **29**, 229-241.e6 (2019).

136. Thouvenin, O. *et al.* Origin and role of the cerebrospinal fluid bidirectional flow in the central canal. *eLife* **9**, e47699 (2020).

137. Kramer-Zucker, A. G. *et al.* Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis. *Development* **132**, 1907–1921 (2005).

138. Grimes, D. T. *et al.* Zebrafish models of idiopathic scoliosis link cerebrospinal fluid flow defects to spine curvature. *Science* **352**, 1341–1344 (2016).

139. Lu, H., Shagirova, A., Goggi, J. L., Yeo, H. L. & Roy, S. Reissner fibre-induced urotensin signalling from cerebrospinal fluid-contacting neurons prevents scoliosis of the vertebrate spine. *Biol. Open* **9**, bio052027 (2020).

140. Cantaut-Belarif, Y. *et al.* Adrenergic activation modulates the signal from the Reissner fiber to cerebrospinal fluid-contacting neurons during development. *eLife* **9**, e59469 (2020).

141. Rose, C. D. *et al.* SCO-Spondin Defects and Neuroinflammation Are Conserved Mechanisms Driving Spinal Deformity across Genetic Models of Idiopathic Scoliosis. *Curr. Biol.* **30**, 2363-2373.e6 (2020).

142. Vesque, C. *et al.* Loss of the Reissner Fiber and increased URP neuropeptide signaling underlie scoliosis in a zebrafish ciliopathy mutant. 2019.12.19.882258 Preprint at https://doi.org/10.1101/2019.12.19.882258 (2019).

143. Dai, Z. *et al.* Novel Mutations in UTS2R are Associated with Adolescent Idiopathic Scoliosis in the Chinese Population. *Spine* **46**, E288–E293 (2021).

144. Xie, H. *et al.* Ependymal polarity defects coupled with disorganized ciliary beating drive abnormal cerebrospinal fluid flow and spine curvature in zebrafish. *PLOS Biol.* **21**, e3002008 (2023).

145. Gene [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004 - Gene ID: 23145 SSPOP SCO-spondin, pseudogene [Homo sapiens (human)]; [cited 2023 Jun 06]. https://www.ncbi.nlm.nih.gov/gene/23145.

146. Nair, A., Azatian, G. & McHenry, M. J. The kinematics of directional control in the fast start of zebrafish larvae. *J. Exp. Biol.* **218**, 3996–4004 (2015).

147. Bagnall, M. W. & McLean, D. L. Modular organization of axial microcircuits in zebrafish. *Science* **343**, 197–200 (2014).

148. O'Donovan, M. J., Bonnot, A., Wenner, P. & Mentis, G. Z. Calcium imaging of network function in the developing spinal cord. *Cell Calcium* **37**, 443–450 (2005).

149. Kreutzberger, A. J. B. *et al.* In vitro fusion of single synaptic and dense core vesicles reproduces key physiological properties. *Nat. Commun.* **10**, 3904 (2019).

150. Bonfanti, L. & Seki, T. The PSA-NCAM-Positive 'Immature' Neurons: An Old Discovery Providing New Vistas on Brain Structural Plasticity. *Cells* **10**, 2542 (2021).

151. Wang, S. *et al.* The Neural Stem Cell Properties of PKD2L1+ Cerebrospinal Fluid-Contacting Neurons in vitro. *Front. Cell. Neurosci.* **15**, 630882 (2021).

152. Cao, L. *et al.* The neural stem cell properties of Pkd2l1+ cerebrospinal fluid-contacting neurons in vivo. *Front. Cell. Neurosci.* **16**, 992520 (2022).

153. Kathe, C. *et al.* The neurons that restore walking after paralysis. *Nature* **611**, 540–547 (2022).

154. Le Pichon, C. E. & Chesler, A. T. The functional and anatomical dissection of somatosensory subpopulations using mouse genetics. *Front. Neuroanat.* **8**, 21 (2014).

155. Staurengo-Ferrari, L., Deng, L. & Chiu, I. M. Interactions between nociceptor sensory neurons and microbial pathogens in pain. *Pain* **163**, S57–S68 (2022).

156. Goulding, M. Circuits controlling vertebrate locomotion: moving in a new direction. *Nat. Rev. Neurosci.* **10**, 507–518 (2009).

157. O'Donovan, M. J. *et al.* Imaging the spatiotemporal organization of neural activity in the developing spinal cord. *Dev. Neurobiol.* **68**, 788–803 (2008).

158. Whelan, P. J. Developmental aspects of spinal locomotor function: insights from using the in vitro mouse spinal cord preparation. *J. Physiol.* **553**, 695–706 (2003).

159. Cuellar, C. A. *et al.* Propagation of sinusoidal electrical waves along the spinal cord during a fictive motor task. *J. Neurosci. Off. J. Soc. Neurosci.* **29**, 798–810 (2009).

160. Caggiano, V., Sur, M. & Bizzi, E. Rostro-caudal inhibition of hindlimb movements in the spinal cord of mice. *PloS One* **9**, e100865 (2014).

161. Lun, M. P. *et al.* Spatially Heterogeneous Choroid Plexus Transcriptomes Encode Positional Identity and Contribute to Regional CSF Production. *J. Neurosci.* **35**, 4903–4916 (2015).

162. Engelhardt, B. *et al.* Vascular, glial, and lymphatic immune gateways of the central nervous system. *Acta Neuropathol. (Berl.)* **132**, 317–338 (2016).

163. Xie, L. *et al.* Sleep Drives Metabolite Clearance from the Adult Brain. *Science* **342**, 373–377 (2013).

164. Faghih, M. M. & Keith Sharp, M. Mechanisms of tracer transport in cerebral perivascular spaces. *J. Biomech.* **118**, 110278 (2021).

165. Raghunandan, A. *et al.* Bulk flow of cerebrospinal fluid observed in periarterial spaces is not an artifact of injection. *eLife* **10**, e65958 (2021).

166. Bedussi, B., Almasian, M., de Vos, J., VanBavel, E. & Bakker, E. N. Paravascular spaces at the brain surface: Low resistance pathways for cerebrospinal fluid flow. *J. Cereb. Blood Flow Metab.* **38**, 719–726 (2018).

167. Mestre, H. *et al.* Flow of cerebrospinal fluid is driven by arterial pulsations and is reduced in hypertension. *Nat. Commun.* **9**, 4878 (2018).

168. Smith, A. J., Yao, X., Dix, J. A., Jin, B.-J. & Verkman, A. S. Test of the 'glymphatic' hypothesis demonstrates diffusive and aquaporin-4-independent solute transport in rodent brain parenchyma. *eLife* **6**, e27679 (2017).

169. Smith, A. J. & Verkman, A. S. The “glymphatic” mechanism for solute clearance in Alzheimer’s disease: game changer or unproven speculation? *FASEB J.* **32**, 543–551 (2018).

170. Croci, M., Vinje, V. & Rognes, M. E. Uncertainty quantification of parenchymal tracer distribution using random diffusion and convective velocity fields. *Fluids Barriers CNS* **16**, 32 (2019).

171. Keith Sharp, M., Carare, R. O. & Martin, B. A. Dispersion in porous media in oscillatory flow between flat plates: applications to intrathecal, periarterial and paraarterial solute transport in the central nervous system. *Fluids Barriers CNS* **16**, 13 (2019).

172. Veluw, S. J. van *et al.* Vasomotion as a Driving Force for Paravascular Clearance in the Awake Mouse Brain. *Neuron* **105**, 549-561.e5 (2020).

173. Vinje, V., Eklund, A., Mardal, K.-A., Rognes, M. E. & Støverud, K.-H. Intracranial pressure elevation alters CSF clearance pathways. *Fluids Barriers CNS* **17**, 29 (2020).

174. Kedarasetti, R. T., Drew, P. J. & Costanzo, F. Arterial pulsations drive oscillatory flow of CSF but not directional pumping. *Sci. Rep.* **10**, 10102 (2020).

175. Spector, R., Robert Snodgrass, S. & Johanson, C. E. A balanced view of the cerebrospinal fluid composition and functions: Focus on adult humans. *Exp. Neurol.* **273**, 57–68 (2015).

176. Vijayakrishnan Nair, V. *et al.* Human CSF movement influenced by vascular low frequency oscillations and respiration. *Front. Physiol.* **13**, 940140 (2022).

177. Lewis, L. D. The interconnected causes and consequences of sleep in the brain. *Science* **374**, 564–568 (2021).

178. Fultz, N. E. *et al.* Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. *Science* **366**, 628–631 (2019).

179. Damkier, H. H., Brown, P. D. & Praetorius, J. Cerebrospinal fluid secretion by the choroid plexus. *Physiol. Rev.* **93**, 1847–1892 (2013).

180. Ghersi-Egea, J.-F. *et al.* Molecular anatomy and functions of the choroidal blood-cerebrospinal fluid barrier in health and disease. *Acta Neuropathol. (Berl.)* **135**, 337–361 (2018).

181. Korzh, V. Development of the brain ventricular system from a comparative perspective. *Clin. Anat. N. Y. N* **36**, 320–334 (2023).

182. Reiter, R. J., Tan, D. X., Kim, S. J. & Cruz, M. H. C. Delivery of pineal melatonin to the brain and SCN: role of canaliculi, cerebrospinal fluid, tanycytes and Virchow–Robin perivascular spaces. *Brain Struct. Funct.* **219**, 1873–1887 (2014).

183. *The Subcommissural Organ*. (Springer, 1993). doi:10.1007/978-3-642-78013-4.

184. Reissner, E. Beiträge zur Kenntnis vom Bau des Rückenmarkes von Petromyzon fluviatilis L. *Arch Anat Physiol Wiss Med Leipz.* **1**, 545–588 (1860).

185. Sepúlveda, V., Maurelia, F., González, M., Aguayo, J. & Caprile, T. SCO-spondin, a giant matricellular protein that regulates cerebrospinal fluid activity. *Fluids Barriers CNS* **18**, 45 (2021).

186. Dani, N. *et al.* A cellular and spatial map of the choroid plexus across brain ventricles and ages. *Cell* **184**, 3056-3074.e21 (2021).

187. Zappaterra, M. D. *et al.* A Comparative Proteomic Analysis of Human and Rat Embryonic Cerebrospinal Fluid. *J. Proteome Res.* **6**, 3537–3548 (2007).

188. Fame, R. M. & Lehtinen, M. K. Emergence and Developmental Roles of the Cerebrospinal Fluid System. *Dev. Cell* **52**, 261–275 (2020).

189. Xu, H. *et al.* Choroid plexus NKCC1 mediates cerebrospinal fluid clearance during mouse early postnatal development. *Nat. Commun.* **12**, 447 (2021).

190. Assialioui, A., Domínguez, R., Ferrer, I., Andrés-Benito, P. & Povedano, M. Elevated Cerebrospinal Fluid Proteins and Albumin Determine a Poor Prognosis for Spinal Amyotrophic Lateral Sclerosis. *Int. J. Mol. Sci.* **23**, 11063 (2022).

191. Sjoqvist, S. & Otake, K. A pilot study using proximity extension assay of cerebrospinal fluid and its extracellular vesicles identifies novel amyotrophic lateral sclerosis biomarker candidates. *Biochem. Biophys. Res. Commun.* **613**, 166–173 (2022).

192. Dreger, M., Steinbach, R., Otto, M., Turner, M. R. & Grosskreutz, J. Cerebrospinal fluid biomarkers of disease activity and progression in amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* **93**, 422–435 (2022).

193. Nemeroff, C. B. *et al.* Elevated Concentrations of CSF Corticotropin-Releasing Factor-Like Immunoreactivity in Depressed Patients. *Science* **226**, 1342–1344 (1984).

194. Seo, J.-S., Mantas, I., Svenningsson, P. & Greengard, P. Ependymal cells-CSF flow regulates stress-induced depression. *Mol. Psychiatry* **26**, 7308–7315 (2021).

195. Piehl, N. *et al.* Cerebrospinal fluid immune dysregulation during healthy brain aging and cognitive impairment. *Cell* **185**, 5028-5039.e13 (2022).

196. Ma, Y., Brettschneider, J. & Collingwood, J. F. A Systematic Review and Meta-Analysis of Cerebrospinal Fluid Amyloid and Tau Levels Identifies Mild Cognitive Impairment Patients Progressing to Alzheimer's Disease. *Biomedicines* **10**, 1713 (2022).

197. Halbgebauer, S. *et al.* CSF levels of SNAP-25 are increased early in Creutzfeldt-Jakob and Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* jnnp-2021-328646 (2022) doi:10.1136/jnnp-2021-328646.

198. Eh, K. *et al.* Update on CSF Biomarkers in Parkinson's Disease. *Biomolecules* **12**, (2022).

199. Barschke, P. *et al.* Cerebrospinal fluid levels of proenkephalin and prodynorphin are differentially altered in Huntington's and Parkinson's disease. *J. Neurol.* **269**, 5136–5143 (2022).

200. Bothwell, S. W., Janigro, D. & Patabendige, A. Cerebrospinal fluid dynamics and intracranial pressure elevation in neurological diseases. *Fluids Barriers CNS* **16**, 9 (2019).

201. Chau, K. F. *et al.* Progressive Differentiation and Instructive Capacities of Amniotic Fluid and Cerebrospinal Fluid Proteomes following Neural Tube Closure. *Dev. Cell* **35**, 789–802 (2015).

202. Fame, R. M., Shannon, M. L., Chau, K. F., Head, J. P. & Lehtinen, M. K. A concerted metabolic shift in early forebrain alters the CSF proteome and depends on MYC downregulation for mitochondrial maturation. *Development* **146**, dev182857 (2019).

203. Holth, J. K. *et al.* The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans. *Science* **363**, 880–884 (2019).

204. Saunders, N. R., Dziegielewska, K. M., Fame, R. M., Lehtinen, M. K. & Liddelow, S. A. The choroid plexus: a missing link in our understanding of brain development and function. *Physiol. Rev.* **103**, 919–956 (2023).

205. Sawamoto, K. *et al.* New Neurons Follow the Flow of Cerebrospinal Fluid in the Adult Brain. *Science* **311**, 629–632 (2006).

206. Lim, D. A. & Alvarez-Buylla, A. The Adult Ventricular-Subventricular Zone (V-SVZ) and Olfactory Bulb (OB) Neurogenesis. *Cold Spring Harb. Perspect. Biol.* **8**, a018820 (2016).

207. Duquenne, M. *et al.* Leptin brain entry via a tanycytic LepR-EGFR shuttle controls lipid metabolism and pancreas function. *Nat. Metab.* **3**, 1071–1090 (2021).

208. Clayton, R. W., Lovell-Badge, R. & Galichet, C. The Properties and Functions of Glial Cell Types of the Hypothalamic Median Eminence. *Front. Endocrinol.* **13**, 953995 (2022).

209. Welch, M. J., Markham, C. H. & Jenden, D. J. Acetylcholine and choline in cerebrospinal fluid of patients with Parkinson's disease and Huntington's chorea. *J. Neurol. Neurosurg. Ampmathsemicolon Psychiatry* **39**, 367–374 (1976).

210. Strittmatter, M. *et al.* Cerebrospinal Fluid Neuropeptides and Monoaminergic Transmitters in Patients With Trigeminal Neuralgia. *Headache J. Head Face Pain* **37**, 211–216 (1997).

211. Gabelle, A. *et al.* Cerebrospinal fluid levels of orexin-A and histamine, and sleep profile within the Alzheimer process. *Neurobiol. Aging* **53**, 59–66 (2017).

212. Orhan, F. *et al.* CSF GABA is reduced in first-episode psychosis and associates to symptom severity. *Mol. Psychiatry* **23**, 1244–1250 (2017).

213. Spreux-Varoquaux, O. *et al.* Glutamate levels in cerebrospinal fluid in amyotrophic lateral sclerosis: a reappraisal using a new HPLC method with coulometric detection in a large cohort of patients. *J. Neurol. Sci.* **193**, 73–78 (2002).

214. Martin, J. *et al.* Vasopressin and oxytocin in CSF and plasma of patients with aneurysmal subarachnoid haemorrhage. *Neuropeptides* **48**, 91–96 (2014).

215. Nilsson, C. L., Brinkmalm, A., Minthon, L., Blennow, K. & Ekman, R. Processing of neuropeptide Y, galanin, and somatostatin in the cerebrospinal fluid of patients with Alzheimer's disease and frontotemporal dementia. *Peptides* **22**, 2105–2112 (2001).

216. Veening, J. G. & Barendregt, H. P. The regulation of brain states by neuroactive substances distributed via the cerebrospinal fluid; a review. *Cerebrospinal Fluid Res.* **7**, 1 (2010).

217. Kandel, E. R., Schwartz, J. & Jessell, T. *Principles of Neural Science, Fourth Edition*. (McGraw-Hill Companies,Incorporated, 2000).

218. Hudspeth, A. J. Integrating the active process of hair cells with cochlear function. *Nat. Rev. Neurosci.* **15**, 600–614 (2014).

219. Pepermans, E. & Petit, C. The tip-link molecular complex of the auditory mechano-electrical transduction machinery. *Hear. Res.* **330**, 10–17 (2015).

220. Pan, B. *et al.* TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear. *Neuron* **79**, 504–515 (2013).

221. Steiner, A. B., Kim, T., Cabot, V. & Hudspeth, A. J. Dynamic gene expression by putative hair-cell progenitors during regeneration in the zebrafish lateral line. *Proc. Natl. Acad. Sci. U. S. A.* **111**, E1393-1401 (2014).

222. Desban, L. *et al.* Lateral line hair cells integrate mechanical and chemical cues to orient navigation. 2022.08.31.505989 Preprint at https://doi.org/10.1101/2022.08.31.505989 (2022).

223. Naseri Kouzehgarani, G. *et al.* Harnessing cerebrospinal fluid circulation for drug delivery to brain tissues. *Adv. Drug Deliv. Rev.* **173**, 20–59 (2021).

224. Böhm, U. L. & Wyart, C. Spinal sensory circuits in motion. *Curr. Opin. Neurobiol.* **41**, 38–43 (2016).

Acknowledgements

This work has received support from the European Research Council (ERC), the New York Stem Cell Foundation (NYSCF), the Human Frontier Science Program (HFSP), the Fondation pour la Recherche Médicale (FRM), the Fondation Bettencourt-Schueller (FBS), the Fondation Schlumberger pour l'Education et la Recherche (FSER), and the Marie Skłodowska-Curie European Training Network initiative. We thank Headquarter for conceiving illustrations, J. Guedes, manager of the Zenith program, for feedback and all members of the Wyart lab past and present in the Paris Brain Institute (Institut du Cerveau).

Author contributions

C.W. researched data for the article and wrote the article. C.W., M. C-T., Y. C-B. and A. O-D. contributed substantially to discussion of the content. C.W. and U.L.B. reviewed and/or edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Supplementary information

Supplementary information is available for this paper at https://doi.org/10.1038/s415XX-XXX-XXXX-X

….

Fig. 1: Overview of cerebrospinal fluid-contacting neuron (CSF-cN) anatomy and physiological roles. Interoceptive cells at the interface with the cerebrospinal fluid (CSF), known as CSF-cNs, complement the peripheral propriosensory system by providing information on the curvature of the spine that can regulate locomotion, posture and morphogenesis**. a,** in vertebrate species, CSF is largely produced by the choroid plexuses and flows in and around the CNS. CSF circulates in a specific direction (indicated by the arrows) through the brain ventricles, the subarachnoid spaces and the central canal of the spinal cord[161].. **b**, In the periphery, dorsal root ganglia (DRG) somatosensory neurons detect forces related to muscle contractions, tension on Golgi tendon organs or mechanical forces applied on the skin. In mammals, specialized structures (muscle spindles and Golgi tendon organs) detect forces related to muscle contraction and this information is conveyed by the DRG peripheral axon to motor neurons and premotor interneurons in the spinal cord[4,8,46,52,74]. In contrast, in fish (not shown), the peripheral axons of DRG neurons and spinal mechanosensory cells called Rohon-Beard neurons have endings terminating under the skin without forming muscle spindles. **c**, Within the spinal cord, CSF-cNs detect spinal curvature on the side of compression[4] through a functional interaction with the Reissner fibre. In addition, CSF-cNs detect changes in the concentration of biomolecules in the CSF that flows in the central canal[19]. **d,** Schematic summary of the roles of CSF-cNs. In fish and mammals, the sensory and neurosecretory functions of spinal CSF-cNs constitute an avenue to regulate locomotion, posture, spine morphogenesis and innate immunity. CSF-cNs, together with the Reissner fibre, constitute a multimodal intraspinal sensory system that is involved in mechanosensation, both on the timescale of milliseconds (to optimize speed of locomotion and keep balance) and on the timescale of days and weeks (to straighten the body axis and maintain the proper curvature of the spine)[6,7,79,141]. This axial sensory system also responds to CSF changes in pH and osmolarity as well as to bacterial metabolites in the context of an infection[19]. Upon acute activation, CSF-cNs release GABA and somatostatin to modulate the activity of their targets within the spinal motor circuits[5,11,17,46,52,74] and can also act long-distance by changing the content of the CSF. Upon sustained activation, CSF-cNs can release peptides of the urotensin family[15,16] that act to straighten the body axis and the spine during development and in ageing. Upon activation during an infection of the central nervous system, CSF-cNs can secrete diverse compounds such as cytokines and complement factors in order to enhance survival of the host as well as peptides that trigger dorsal arching[19]. Part a is adapted, with permission, from REF[223]

Fig. 2: Cerebrospinal fluid-contacting neurons (CSF-cNs) respond to compression during spinal bends. a-b, In fish, during lateral bends of the spinal cord towards either the left **(a)** or the right **(b)** side of the animal, dorsolateral CSF-cNs are activated on the concave side of the spinal cord[4]**. c,** Ventral CSF-cNs are recruited by longitudinal bends in the sagittal plane[74]. It is hypothesized that compression resulting from negative curvature can be detected in the central canal by the relative movement of the Reissner fibre towards the wall of the canal where CSF-cNs are inserted and that this movement is sensed by the CSF-cNs via their apical extension[7]. Schematics represent findings reported in REFs [7,64,67,224]. Figure adapted, with permission, from REF[7].

Fig. 3: Diverse chemosensory functions of cerebrospinal fluid-contacting neurons (CSF-cNs). **a,** CSF-cNs detect acidification and alkalinization of the CSF via acid sensing ion channels (ASIC channels)[11] and polycystic kidney disease 2-like 1 (PKD2L1) channels[10]. **B,** When live pathogenic bacteria, such as *Streptococcus pneumoniae*, invade the CSF, CSF-cNs detect bacterial metabolites via multiple orphan taste receptors that, upon activation, lead to a massive calcium entry in the cell[19]. During such an infection, CSF-cNs contribute to innate immunity by expressing and releasing various peptides and proteins (including complement factors, cytokines, neuropeptides and factors attracting macrophages)[19].

Fig 4: Connectivity of cerebrospinal fluid-contacting neurons (CSF-cNs) in zebrafish. CSF-cNs project an axon that ascends ipsilaterally in the spinal cord (and, for the rostral most CSF-cNS, extends into the hindbrain[2]). In the spinal cord, ventral CSF-cNs project onto primary 'fast' motor neurons involved in postural control and fast swimming[74] and dorsolateral CSF-cNs project onto V0-v premotor interneurons[52]. Ventral and dorsolateral CSF-cNs also project onto giant sensory interneurons that send back a corollary discharge to the brainstem[74]. In the hindbrain, the rostral most CSF-cNs synapse onto the soma of occipital motor neurons and make synapses onto the descending fibres from visual system homeobox 2 (Vsx2)-expresssing reticulospinal neurons (also known as V2a neurons) and neuromodulatory serotonergic fibres. Schematics based on findings reported in REFs [42,46,52,74,115].... In mice (not fully depicted here in this schematic due to space constraints) , CSF-cNs project onto themselves[51,52], onto motor neurons of axial muscles in the medial motor column and onto glutamatergic premotor interneurons such as V0-c and V2a interneurons[51,52]. Neurosecretion in CSF-cNs is assumed to occur in the CSF via the apical extension, although this has not been rigorously investigated and is therefore not shown here.

Fig. 5: Molecular mechanisms linking the cerebrospinal fluid sensory interface and morphogenesis throughout life. Wild type (control) zebrafish develop a straight body axis at the embryonic, juvenile and adult stage. In SCO-spondin (*sspo*) knock-out or hypomorph zebrafish, the Reissner fibre does not form and the embryo develops a curled down axis[6]. In zebrafish mutants in which the Reissner fibre is not maintained at the juvenile stage, the fish develops a 3D torsion of the spine[79,141] that is reminiscent of idiopathic scoliosis in humans. Mutant zebrafish lacking both urotensin-related peptide 1 (Urp1) and Urp2, or those that lack the receptor for these peptides

(Uts2r3, expressed in dorsal skeletal muscles), grow with a straight body axis at the embryonic stage but show 3D deformations of the spine at the juvenile stages and beyond[16]. Zebrafish carrying mutations in the polycystic kidney disease 2-like 1 (Pkd2l1) channel show in late-adulthood a 2D deformation of the spine, reminiscent of kyphosis in humans[67].

Box 1: Cerebrospinal fluid (CSF): a rich signaling route for brain–body interactions.

CSF is produced via secretion in the ventricular space from the epithelium of the choroid plexuses[161], while CSF drainage occurs via lymphatic vessels towards the blood and the lymphatic system[162]. CSF flows around the CNS in the perivascular spaces and its movement through periarterial spaces in particular can contribute to the clearance of metabolic waste products[163]. In mice, contradictory results were obtained in experiments designed to quantify CSF flow, leading to questions about whether the injection of particles may lead to artifacts via the addition of fluid that increases intra cranial pressure [164–174]. However, recent evidence indicates that pulsatile CSF flow (occurring as a bulk flow that is aligned with blood flow) does occur in the perivascular spaces surrounding pial arteries, even when tracer-particles are infused without the addition of fluid[165]. CSF flow is in dynamic flux with heartbeat, posture and respiration[175,176]. CSF flow is coupled to neuronal activity and therefore influenced by circadian rhythms and sleep[163,177]: it oscillates as a function of local hemodynamic oscillations in the brain that follow neural slow waves [178].

Changes in CSF composition can occur either rapidly (through the selective transcytosis of blood-borne factors) or slowly (through de novo protein synthesis and secretion from epithelial cells)[161,179,180]. Changes in the CSF content can also result from the activity of diverse conserved neurosecretory structures[181] located at the interface with the CSF, such as CSF-contacting neurons (CSF-cNs) in the brain ventricles and spinal cord[22,72] or secretory epithelial cells in the circumventricular organs. The circumventricular organs are conserved in vertebrates[181] and, in addition to the choroid plexuses, include the pineal gland[182], the paraventricular organ, the subfornical organ, the median eminence, the neurohypophysis, the area postrema and the subcommissural organ (SCO)[183]. The epithelium of the SCO secretes the large SCO-spondin glycoprotein that aggregates into an acellular and extracellular thread called the Reissner fibre, which runs from the third ventricle to the caudal end of the central canal in the spinal cord[184,185].

Despite this remarkable polymer being discovered over 160 years ago, *scospondin* (also referred to as *'sspo')* zebrafish mutants have been engineered only recently using CRISPR-Cas genome-editing technologies [6,79,139–141]. This work revealed an essential role for the Reissner fibre in the alignment of the body axis and spine [6,79,139–141]. The Reissner fibre has numerous binding sites for biomolecules present in the CSF and could contribute to setting the CSF's activity and properties (such as its osmolarity or biomolecular composition) [185]. In mammals, CSF secretion from the choroid plexuses differs between the different brain ventricles and across developmental stages and ages[186] resulting in slow changes in CSF composition throughout embryonic and postnatal life[187–189].

In medicine, the analysis of CSF content is classically used to detect pathogens associated with CNS infection or to diagnose neurological diseases such as amyotrophic lateral sclerosis[190–192], depression[193,194], Alzheimer disease[195–197], Hungtington disease and Parkinson disease[198,199]. The CSF has long been seen as a passive fluid, providing nutrients for and ensuring mechanical protection, effective weight reduction and chemical homeostasis of the brain[200]. Yet, the CSF transports biomolecules that are crucial for brain and spine development and activity[201,202] and removes toxic waste[203] and metabolites[163,204]. The circulation of biomolecules generated in the CSF in a particular direction by the beating of polarized cilia in the brain ventricles contributes to neurogenesis in the brain[205,206]. Via the activity of specialized cells contacting the CSF (such as tanycytes, which transport biomolecules from the blood), glucose and hormones such as leptin[207] (which can reach numerous brain areas to mediate its anorexic effect[208]) are distributed. CSF contains numerous other neuro-active substances that can travel long distances and impact the activity of neurons throughout the CNS, including acetylcholine, serotonin, dopamine[209], noradrenaline[210], histamine[211], GABA[212], glutamate[213] and a plethora of neuropeptides (vasopressin, oxytocin[214], and neuropeptide Y[215] among others[216]) for which spinal CSF-cNs have receptors. Given the complexity of CSF chemical signaling, one can postulate that it may serve as a signaling route to communicate with distant neurons, (or even to reach cells throughout the body via the lymphatic and vascular circulation). Recent evidence indicates that CSF-cNs support such a role by releasing peptides in the CSF that act on skeletal muscles cells[6,15,16,18,20,139,140].

Box 2. Comparison of cerebrospinal fluid-contacting neurons (CSF-cNs) to hair cells.

Let's recapitulate how the morphology of a well-known type of exteroceptive ciliated sensory cell, the hair cell, enables it to detect mechanical forces associated with audition and balance and transmit information to downstream interneurons[217,218]. The apical extension of a hair cell is composed of giant actin-based microvilli called stereocilia. These are organized in ranks of increasing heights, with the tallest ones lying adjacent to the microtubule-based cilium known as the kinocilium. This staircase structure has an axis of mirror symmetry that defines the axis of maximal mechanical sensitivity of the hair cell. To convert mechanical forces (displacements of the hair bundle as low as 1nm in amplitude and up to tens of kilohertz in frequency) into electrical signals, stereocilia are coupled via protein filaments called tip links[219] that gate the opening of mechanosensory ion channels[220]. The length of a hair cell's stereocilia inversely scales with its intrinsic tuning frequency range: cells with short stereocilia (~ 1 μm) in the internal ear encode the highest auditory frequencies, while those in the vestibular system with long stereocilia (~ 50μm) detect head motion in the head. Hair cells respond to fast and transient deflections in both directions along their axis of symmetry: deflections of stereocilia toward the kinocilium increase tension on tip links and lead to the opening of potassium channels. The resulting influx of potassium from the endolymph depolarizes the cell and opens voltage-gated calcium channels that drive the calcium-dependent vesicular release of glutamate from the basal side of the cell and thus the excitation of the peripheral terminals of afferent fibres to convey information to the brain. To amplify acoustic signals, to narrow the frequency selectivity and to broaden the ear's dynamic range, the auditory system relies on an active process in cochlear hair cells[218]: intrinsic mobility of the hair bundle, which — when coupled with somatic motility in mammals — allows the entire auditory system to operate near a dynamical instability.

Although the apical extension of CSF-cNs shows a less crystalline structure than that of hair cells, there are similarities in the way that the axial sensory system formed by CSF-cNs and the Reissner fibre detects mechanosensory cues. A recent preprint reports that at rest, the Reissner fibre is under tension, which slightly activates the apical extension of CSF-cNs and thereby leads to resting spontaneous activity[78]. Furthermore, numerous CSF-cNs exhibit an active kinocilium[4,12]. The expression of myosins[19], acting as molecular motors in the hair bundle, together with adhesion proteins, such as protocadherin 15[19] (known in hair cells to form

filaments connecting the stereocilia to the kinocilium[221,222]), suggest that mechanisms similar to those described for the hair bundle of hair cells may be at play in CSF-cNs. The preliminary evidence for interactions between motile cilia and the Reissner fibre raises another question: does the CSF-cN apical extension or its kinocilium interact with elements of the SCO-spondin forming the Reissner fibre? Overall, there are still many missing pieces in our understanding of the mechanosensation mediated by CSF-cNs, compared to what is known about this process in auditory hair cells.

Glossary terms

Axo-axonic synapses: synapses formed from one axon onto another.

Central pattern generators: self-organizing biological neural circuits that produce rhythmic outputs in the absence of rhythmic input.

Choroid plexuses: A network of blood vessels and epithelial cells in the ventricles of the brain that make cerebrospinal fluid.

Corollary discharge: in motor systems, a copy of the movement command that is broadcast to other regions of the brain to warn them of the impending movement.

Dense core secretory vesicles: vesicles that mediate the regulated release of neuropeptides and peptide hormones.

Escape responses: mechanisms by which animals avoid potential predation in response to an aversive stimulus.

Hyperkyphosis: an excessive curvature of the thoracic spine, commonly referred to as hunchback.

Immunohistochemistry: a method that uses antibodies linked to an enzyme or a fluorescent dye to reveal certain antigens in a sample of tissue.

Innate immunity: the initial response of the body to detect invaders such as viruses, bacteria, parasites and toxins, or to sense wounds or trauma.

Interoceptive: relating to sensory functions arising within the body, in the viscera or within the central nervous system itself.

Kinocilium: a short microscopic hairlike vibrating structure found on the surface of certain cells, which either causes currents in the surrounding fluid or generates active movements of a ciliated apical extension in hair cells and CSF-cNs.

Microtubules : components of the cell skeleton that determine the shape of a cell and are involved in different functions including the assembly of mitotic spindle (in dividing cells) and axon extension (in neurons).

Microvilli: microscopic actin-based cellular membrane protrusions that are involved in a wide variety of functions, including absorption, secretion, cellular adhesion and mechanotransduction.

Progenitor domains: territories in which cascades of transcription factors are expressed during development.

Proprioceptive: relative to proprioception, the sense of body position.

Scoliosis: abnormal lateral curvature of the spine showing 3D torsion.

Taste receptors: a type of cellular receptor which facilitates the sensation of taste.

Transcriptomic analyses: methods involving the quantification of transcriptional activity to reveal a global picture of cell function.

Supplementary Video 1 | **Sagittal time series showing the cilium of a ventral CSF-cN in vivo beating in the central canal.** Video was obtained in a 4 day old larval zebrafish and has a 30Hz acquisition rate. The cilium was beating at 7 Hz. Reprinted from reference[4], Springer Nature Ltd.

Supplementary Video 2 | **Time series showing active beating by the cilium of a CSF-cN from a primary zebrafish culture.** The cilium is labeled by an arrow. Adapted from reference[4], Springer Nature Ltd.

Supplementary Video 3 | **3D serial electron microscopy sections reveal the proximity of CSF-cN cilium to the Reissner fibre.** The blue arrow highlights the CSF-cN and the white arrow indicates the cilium, while the red arrow highlights the Reissner fibre. Adapted with permission from reference[7], Elsevier).

Supplementary Video 4 | **CSF-cN calcium activity and cell position during single tail bends corresponding to acoustovestibular escape responses in larval zebrafish**. In order to quantify the activation of CSF-cNs reflected in the increase in intracellular calcium, a correction of the motion artefact is necessary. This was accomplished by calculating the ratio of the signal of the fluorescent sensor GCaMP, used as a reporter of neuronal activity, over the signal of tagRFP, used as a reporter of cell position relative to the focal plane of the two photon microscope. One can appreciate that CSF-cNs only respond to compression on the side of negative curvature during a tail bend. Adapted from reference[4], Springer Nature Ltd).

Supplementary Video 5 | **Artistic animation showing how the Reissner fibre can interact with CSF-cNs to detect bending of the spinal cord on the side of compression.** The video was created based on the results from refs[4,7]. Animation created with the help of Headquarter (https://headquarter.paris).

Supplementary Video 6 | **CSF-cNs show reduced basal activity and sustained massive calcium transients upon injection of *Streptococcus Pneumoniae* into the brain ventricles.** We can appreciate on this video how the basal activity of CSF-cNs is reduced while a subset of CSF-cNs shows unusually-large and long-lasting calcium transients. Reprinted from reference[19] CC BY 4.0 (https://creativecommons.org/licences/by/4.0/).

Supplementary Video 7 | **CSF-cNs in primary cultures respond to cytolysin and bacterial metabolites secreted by *Streptococcus Pneumoniae***. From left to right: negative control showing no response to a pressure-application of aCSF; response to pneumolysin 0.05mg/mL; response to mixture of bitter compounds at 50mM; response to the bitter compound DMDS tested alone at 50mM; response to the other bitter compound 2 pentanone tested alone at 50mM. Reprinted from reference[19] CC BY 4.0 (https://creativecommons.org/licences/by/4.0/).

Supplementary Video 8 | **Deep learning can be used to estimate the probability of rolling probability over time during acoustovestibular responses of larval zebrafish.** By focusing on the head, analysis revealed that, after ablation of CSF-cNs, larval zebrafish roll more often and do so by losing balance after the initial large bend called the C-bend. When rolling is happening, a red dot appears at the top left of the image. The probability for rolling is indicated in the bottom left corner. Reprinted with permission from reference[46], Elsevier).

Supplementary Video 9 | **Dostral CSF-cNs project onto the soma of occipital motor neurons in the hindbrain**. The video shows a dorsal view of the zebrafish hindbrain . CSF-cNs appear in green and motor neurons appear in pink. Synapses are highlighted with arrows. Reprinted with permission from reference[46], Elsevier).

Supplementary Video 10 | **Rostral CSF-cNs form axo-axonic connections onto Vsx2+ reticulospinal neurons in the hindbrain** . The video shows a dorsal view of the zebrafish hindbrain. CSF-cNs appear green and Vsx2-positive reticulospinal neurons appear in pink. Synapses are highlighted with arrows. Reprinted with permission from reference[46], Elsevier).