Throughout vertebrates, cerebrospinal fluid-contacting neurons (CSF-cNs) are ciliated cells surrounding the central canal in the ventral spinal cord. Their contribution to modulate locomotion remains undetermined. Recently, we have shown CSF-cNs modulate locomotion by directly projecting onto the locomotor central pattern generators (CPGs), but the sensory modality these cells convey to spinal circuits and their relevance to innate locomotion remain elusive. Here, we demonstrate in vivo that CSF-cNs form an intraspinal mechanosensory organ that detects spinal bending. By performing calcium imaging in moving animals, we show that CSF-cNs respond to both passive and active bending of the spinal cord. In mutants for the channel Pkd2l1, CSF-cNs lose their response to bending and animals show a selective reduction of tail beat frequency, confirming the central role of this feedback loop for optimizing locomotion. Altogether, our study reveals that CSF-cNs constitute a mechanosensory organ operating during locomotion to modulate spinal CPGs.
behavior has long been known to be modulated by the content of cerebrospinal fluid (CSF)\textsuperscript{1,2}. Studies of CSF flow have shown that locomotor defects observed in normal pressure hydrocephalus can be reversed by CSF elimination. More recently, CSF has been shown to relay important clues to migrating neurons during development\textsuperscript{3,4} and to remove metabolites during sleep\textsuperscript{5}. However, the mechanism by which molecular and hydrodynamic properties of the CSF change network dynamics is unknown. In the vertebrate spinal cord, a unique and conserved population of GABAergic neurons extends microvilli into the CSF\textsuperscript{6–10}. These neurons, referred to as CSF-cNs (and in *Xenopus* and *Danio rerio* as KA neurons\textsuperscript{11}), are among the most poorly understood components of the vertebrate motor network. We previously showed that CSF-cNs are among the most poorly understood components of the vertebrate motor network. We previously showed that CSF-cNs exhibit a motile kinocilium and multiple microvilli. We used a promoter of *pkd2l1* that specifically targets CSF-cNs in the larval spinal cord (Fig. 1a, out of 253 *pkd2l1*\textsuperscript{+} cells from five larvae, CSF-cNs represented 94.2% of total cell counts, see also\textsuperscript{13}) to drive the expression of fluorescent proteins (Fig. 1a–e). Previous studies suggested that CSF-cNs extend cilia into the central canal\textsuperscript{6,9,11,17,18} as depicted in Fig. 1c. We co-labeled CSF-cNs with Arl13b–GFP as a marker of the ciliary axoneme in *Tg(fact:arl13-GFP, pkd2l1:Gal4;cmcl2:eGFP, UAS:tagRFP-CAAX)* triple transgenic larvae (Fig. 1d). We observed a single cilium extending from a brush of microvilli (Fig. 1d′,d″). To establish whether the CSF-cN cilium was motile, we used APEX-2, a genetically-encoded peroxidase, to label CSF-cNs for electron microscopy (Fig. 1e). Our analysis of the ciliary ultrastructure reveals a 9 + 2 organization, indicating that CSF-cNs extend a single motile cilium into the central canal (Fig. 1e′,e″). This was necessary for CSF-cN mechanosensory response to bending. Furthermore, the loss of CSF-cN sensory function leads to a reduction of tail beat frequency in *pkd2l1* mutants as well as in animals with impaired synaptic release from CSF-cNs, confirming the importance of this sensory feedback loop in the regulation of locomotion. Together, our results demonstrate that Pkd2l1 orchestrates a feedback loop that relays information on local curvature of the spinal cord to motor circuits in order to regulate the frequency of locomotion.

**Results**

CSF-cNs exhibit a motile kinocilium and multiple microvilli. We used a promoter of *pkd2l1* that specifically targets CSF-cNs in the larval spinal cord (Fig. 1a, out of 253 *pkd2l1*\textsuperscript{+} cells from five larvae, CSF-cNs represented 94.2% of total cell counts, see also\textsuperscript{13}) to drive the expression of fluorescent proteins (Fig. 1a–e). Previous studies suggested that CSF-cNs extend cilia into the central canal\textsuperscript{6,9,11,17,18} as depicted in Fig. 1c. We co-labeled CSF-cNs with Arl13b–GFP as a marker of the ciliary axoneme in *Tg(fact:arl13-GFP, pkd2l1:Gal4;cmcl2:eGFP, UAS:tagRFP-CAAX)* triple transgenic larvae (Fig. 1d). We observed a single cilium extending from a brush of microvilli (Fig. 1d′,d″). To establish whether the CSF-cN cilium was motile, we used APEX-2, a genetically-encoded peroxidase, to label CSF-cNs for electron microscopy (Fig. 1e). Our analysis of the ciliary ultrastructure reveals a 9 + 2 organization, indicating that CSF-cNs extend a single motile cilium into the central canal (Fig. 1e′,e″). This was necessary for CSF-cN mechanosensory response to bending. Furthermore, the loss of CSF-cN sensory function leads to a reduction of tail beat frequency in *pkd2l1* mutants as well as in animals with impaired synaptic release from CSF-cNs, confirming the importance of this sensory feedback loop in the regulation of locomotion. Together, our results demonstrate that Pkd2l1 orchestrates a feedback loop that relays information on local curvature of the spinal cord to motor circuits in order to regulate the frequency of locomotion.

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**Figure 1** | In the spinal cord, *pkd2l1*\textsuperscript{+} CSF-cNs project an apical extension consisting of one kinocilium and a brush of microvilli into the central canal. (a) Transmitted and fluorescent image showing that the *pkd2l1* promoter in *Tg(pkd2l1:GCaMP5G)* transgenic larva drives GCaMP5G expression in CSF-cNs along the entire spinal cord. Scale bar: 500 \(\mu\)m. (b) A close up from a lateral view in the same transgenic animal shows the morphology of CSF-cNs as elongated cells with an apical extension reaching the central canal. Scale bar: 100 \(\mu\)m. (c) Schematic depicting dorsal and ventral CSF-cN location around the central canal. (d) Confocal microscopy of 50 \(\mu\)m sections of the 4 dpf triple transgenic larva *Tg(pkd2l1:gal4, UAS:tagRFP-CAAX, cmcl2:eGFP, fact:arl13-GFP)* shows that CSF-cNs project one Arl13-GFP + cilium (arrowhead) and multiple microvilli into the CSF. Scale bar: 3 \(\mu\)m. (e) Transmission electron microscopy in *Tg(pkd2l1:gal4)* larvae injected with UAS:APEx2-tagRFP shows a single ventral CSF-cN reaching the central canal (close up in (e′), scale bar: 2 \(\mu\)m) and exhibiting a motile 9 + 2 cilium (arrowhead, (e″), scale bar: 250 nm).
confirmed by video recording of CSF-cNs in vivo (Supplementary Movie 1) as well as in zebrafish dissociated cell cultures (Supplementary Movie 2). The unusual morphology of CSF-cNs may be described as disorganized hair cells located at the interface with the CSF and extending an ascending ipsilateral axon in the spinal cord.

CSF-cNs show minimal activation during fictive escapes. To investigate the involvement of CSF-cNs in the control of locomotion, we characterized their activity patterns during spinal network activity in paralyzed larvae. We used two-photon calcium imaging to record cellular activity during fictive escape behavior induced by a water jet to the otic vesicle (Fig. 2a). Fictive locomotor output was monitored by recording the ventral nerve root (Fig. 2a)19. We compared CSF-cN activity to dorsal motor neurons (MNs), which are known to be active during escape behavior (Fig. 2b)20. MNs showed strong calcium transients during fictive escape behavior (mean $\Delta F/F_{RMS} = 1.2 \pm 0.1$; Fig. 2c top panels and 2d). In contrast, fictive locomotion only led to minute changes in calcium activity in CSF-cNs (mean $\Delta F/F_{RMS} = 0.042 \pm 0.007$; Fig. 2c,d bottom panels). To verify that the lack of activity in CSF-cNs was not due to $\alpha$-bungarotoxin used as a paralytic, we repeated the experiment in $\alpha$-bungarotoxin-treated larvae, where a mutation in the $\beta_{12}$ subunit of the dihydropyridine receptor prevents contraction of skeletal muscle21. The lack of activation of CSF-cNs during fictive escapes in $\alpha$-bungarotoxin-treated larvae can be described as disorganized hair cells located at the interface with the CSF-cNs and extending an ascending ipsilateral axon in the spinal cord.

Muscle contraction leads to strong activation of CSF-cNs. To test whether CSF-cNs are recruited during active locomotion, we performed calcium imaging in the spinal cord of head-restrained, tail-free animals performing escape behaviours (Fig. 3a). We took advantage of the slow decay of GCaMP5G transients in CSF-cNs ($\tau_{G=CSF-cN} = 1.18 \pm 0.07$ s) relative to tail movements (<200 ms) to compare calcium signals before and after active tail motion. TagRFP was co-expressed in CSF-cNs with GCaMP5G to track moving cells and to correct for motion artifacts (see Methods section; Fig. 3a’ and Supplementary Fig. 2). We simultaneously performed high-speed video recording of the entire larva to quantify the behavior in response to otic vesicle stimulation (Supplementary Movie 3). In contrast to paralyzed larvae, we observed strong activation of CSF-cNs in response to tail bends ($\Delta R/R_{G=CSF-cN} = 0.38 \pm 0.08$). Moreover, after unilateral tail bends, our results revealed a clear activation of dorsal CSF-cNs ipsilateral to the contracting side ($\Delta R/R_{G=CSF-cN} = 0.9 \pm 0.2$; Fig. 3b) whereas dorsal contralateral and ventral cells remained mostly silent ($\Delta R/R_{G=CSF-cN} = 0.20 \pm 0.08$; $\Delta R/R_{G=CSF-cN} = 0.10 \pm 0.07$; Fig. 3b). Our data indicate that dorsal CSF-cNs respond to active bending of the spinal cord selectively on the contracting side.

Bending of the tail recruits CSF-cNs in paralyzed animals. To test whether CSF-cNs respond to mechanical stimulation during bending of the spinal cord, we mimicked the mechanical deformation associated with muscle contraction by imposing a local bend on the tail of paralyzed larvae using a mechanical probe. The tail of agarose-embedded larvae was exposed on one side and pressed with a glass probe (Fig. 3c). Here, CSF-cNs

Figure 2 | CSF-cNs are minimally activated during fictive escapes when no muscle contraction occurs. (a) Schematic view of the experimental setup combining 2-photon laser scanning microscope for calcium imaging and electrophysiological recordings of the ventral nerve root. 10 ms-long water jets delivered to the otic vesicle triggered fictive escapes in paralyzed larvae. (b) Lateral view showing expression of GCaMP6F in MNs in the double transgenic larva Tg(mnx1:Gal4, UAS:GCaMP6F;cytae3:Cherry) and in CSF-cNs in Tg(pkdw21:gal4, UAS:GCaMP6F;cytae3:Cherry). ROIs indicate cells included in the analysis. Only the dorsalmost MNs were analyzed in the mnx1 line. Scale bar: 20 $\mu$m. (c) Typical calcium transients recorded in MNs (blue) and in CSF-cNs (red) during fictive escapes; ‘stimulus’ indicates when the water jet was triggered; average response in coloured lines. (d) Quantification of calcium transient amplitude in MNs and CSF-cNs (each data point represents one recording from one cell; plots use median as the measure of central tendency; inset is the cumulative histogram of calcium responses). Responses in both populations are greater than baseline (204 MNs from 6 larvae: mean $\Delta F/F = 1.2, P < 10^{-8}$; 192 CSF-cNs from 7 larvae: mean $\Delta F/F = 0.042, P = 3.78 \times 10^{-8}$), but CSF-cNs exhibit significantly less activity than MNs ($P = 0.014$).
showed activity in response to mechanical stimulation (ΔR/R_{CSF-cN} = 1.0 ± 0.1; Fig. 3d). The response was restricted to the site of stimulation and its amplitude decreased with increasing distance from the probe (Fig. 3e). Dorsal CSF-cNs ipsilateral to the stimulation site showed the largest responses (ΔR/R_{CSF-cN} = 1.5 ± 0.3; Fig. 3f) while dorsal contralateral and ventral CSF-cNs showed a significant, albeit much smaller, response (ΔR/R_{CSF-cN} = 0.5 ± 0.2; ΔR/R_{CSF-cN} = 0.8 ± 0.1; Fig. 3f). Results from passive bending further support the hypothesis that CSF-cNs constitute an in vivo mechanosensory system locally detecting bending of the spinal cord.

**Bending-evoked responses are abolished in pkd2l1 mutants.** The restricted expression of PKD2L1 to CSF-cNs in the spinal cord and its conservation across multiple vertebrate species suggest an important role specific to CSF-cNs. To test whether this channel contributes to the mechanical response of CSF-cNs, we used transcription activator-like effector nucleases (TALENs) to generate a pkd2l1 null mutant (Fig. 4a). CSF-cNs without functional Pkd2l1 show no overall morphological defects in zebrafish larva and their cilia were still beating in vitro (n = 3). However, the responses of dorsal ipsilateral CSF-cNs to active bending (pkd2l1^{icm02/icm02} ΔR/R_{CSF-cN} = 0.07 ± 0.06; Fig. 4b) and passive bending (pkd2l1^{icm02/icm02} ΔR/R_{CSF-cN} = 0.03 ± 0.06; Fig. 4c) abolished in pkd2l1^{icm02/icm02} mutants. Our data indicate a crucial role of Pkd2l1 in mediating CSF-cN detection of spinal bending in zebrafish larvae.

**pkd2l1 mutants show reduced tailbeat frequency.** The ultimate output of sensory processing in the central nervous system is behavior. To probe the active contribution of the CSF-cN sensory motor loop to locomotion, we quantified acoustically-induced escape behavior in pkd2l1^{icm02/icm02} mutants, where CSF-cN response to bending is impaired (457 escapes from 141 larvae, seven experiments, Fig. 4d). Kinematic analysis revealed a specific decrease of mean tail beat frequency (TBF) in pkd2l1^{icm02/icm02} mutants compared to WT siblings (32.8 ± 0.6 Hz versus 35.2 ± 0.7 Hz; Fig. 4d and Supplementary Fig. 3a). We observed a significant effect of trial number on TBF (Fig. 4f), latency, distance, speed and number of oscillations (Supplementary Fig. 3b, c).

![Figure 3](https://example.com/figure3.png)

**Figure 3 | CSF-cNs respond to active muscle contraction as well as to passive mechanical bending of the spinal cord.** (a) Schematic describing 2-photon imaging experiments used to record simultaneously from CSF-cNs expressing tagRFP (magenta) and GCaMPs (green) in head-embedded Tg(pkdl2l1:GCaMP5, pkdl2l1:tagRFP) larvae. Infrared illumination combined with high-speed video recording shows unidirectional tail deflections induced by a water jet to the otic vesicle. Sample traces for ΔF/ of tagRFP and GCaMP shown with tail deflection during escape (note: vertical scale is 10 times larger for GCaMP compared to tagRFP signals). Subtracting the tagRFP signal from the GCaMP signal removed motion artifacts; breaks in the trace arise from frames when cells escaped from the focal plane. Scale bar: 10 μm. (b) Quantification of calcium transient amplitude in response to muscle contraction (n = 11 larvae) in dorsal CSF-cNs either ipsilateral (red, 31 cells) or contralateral (yellow, 19 cells) or ventral (purple, 44 cells). Only dorsal ipsilateral cells exhibited responses greater than baseline (P = 9.51 × 10^{-6}) and all other cell types responded significantly less than dorsal ipsilateral cells (dorsal contralateral: P = 2.43 × 10^{-3}, ventral: P = 4.85 × 10^{-5}). (c) Passive mechanical stimulation of CSF-cNs in paralyzed larvae (n = 5) was implemented with mechanical pressure exerted by pushing a glass probe laterally against the fish tail. Scale bar: 50 μm. (d) Response of proximal (<100 μm) and distal (>100 μm) CSF-cNs. Inset: average calcium response of proximal (red) versus distal (blue) CSF-cNs. (e) Response of dorsal ipsilateral (red) CSF-cNs as function of distance from the probe. (f) Response of dorsal ipsilateral (red, 28 cells), dorsal contralateral (yellow, 16 cells) and ventral (purple, 36 cells) CSF-cNs relative to the location of mechanical stimulation. Inset: Average calcium response of dorsal ipsilateral versus dorsal contralateral and ventral CSF-cNs. All cell types show a response different from 0 (dorsal ipsilateral: P < 1.0 × 10^{-5}, dorsal contralateral: P = 5.95 × 10^{-5}, ventral: P < 1.0 × 10^{-5}) and all other cell types responded significantly less than dorsal ipsilateral cells (dorsal contralateral: P = 1.06 × 10^{-3}, ventral: P = 7.22 × 10^{-3}).
Fig. 4b) indicating some habituation to stimulus even at an inter trial interval of 2 min. Accounting for this trial effect and using a mixed linear model, we found that TBF was reduced in the pkd2l1icm02/icm02 mutant across trials (Fig. 4f). The specific reduction of TBF in larvae lacking CSF-cN sensory response reveals that CSF-cN feedback is needed to maximize locomotor frequency during active locomotion. We recapitulated this effect by silencing the vesicular release of CSF-cNs with Botulinum toxin light chain in Tg(pkd2l1:gal4;UAS:BoTxBLC-GFP) transgenic larvae (36.3 ± 0.7 Hz versus 38.9 ± 0.5 Hz; Supplementary Fig. 4 (ref. 22)). The convergence of effects obtained from these two independent methods for silencing CSF-cNs demonstrates their central role in the modulation of locomotor frequency.

Discussion
The hypothesis that CSF-cNs constitute a mechanosensitive organ was already formulated by Kolmer, although to-date no experiments had formally addressed this question in vivo. Here, we report activation of CSF-cNs in response to active as well as passive spinal bending (Supplementary Fig. 5). In natural conditions, we show here that CSF-cN mechanosensory response shapes innate locomotion. Although a small population of pkd2l1 expressing neurons was described in dorsal spinal neurons at embryonic stages, this population is undetectable in the larva and therefore unlikely to mediate the behavioural effects presented here (see Supplementary Fig. 1 of reference 13).

Whether CSF-cNs are the direct mechanoreceptors or whether they receive input from other, unidentified mechanosensitive cells remains to be determined. CSF-cNs may also respond to chemical cues released in the central canal as a response to mechanical stimulation or muscle contraction. Although we cannot exclude a chemical hypothesis, it is unclear how a chemical signal would translate into the lateralized mechanoresponse described here. Ciliary calcium signalling may contribute to the sensory response of CSF-cNs as the PKD2L1 channel contributes to ciliary signalling23. PKD2L1 positive CSF-cNs were previously shown to be pH sensitive14,15,24, even though acid sensing ion channels (ASICs) carry most of the proton current14,25,26. Our study here focused on the role of Pkd2l1 as, in the spinal cord, the channel is specific to CSF-cNs. However, as recently shown, PKD2L1 can

![Diagram](image-url)
trigger large calcium spikes upon changes of extracellular calcium, pH or membrane potential. This channel in CSF-cNs may therefore act in concert with other channels such as ASICs contributing to mouse and lamprey CSF-cN response in vitro in order to generate the bending response reported here in vivo.

Previously, we demonstrated that spinal CSF-cNs project directly onto locomotor CPGs involved in slow locomotion. During active locomotion, when muscles contract, we show that CSF-cNs can provide a proprioceptive GABAergic feedback that finely tunes the oscillatory frequency of the locomotor CPG. Further studies linking CSF-cNs to components of the fast locomotor CPG, and the escape circuit in particular, will be necessary to establish the neuronal basis for the modulation we report here. Although sensory feedback is not necessary for the oscillations, there are multiple lines of evidence suggesting that mechanosensory feedback can entrain the rhythm and gate locomotor transitions. The increased frequency of oscillations due to mechanosensory feedback observed here could contribute to the massive reduction of locomotor frequency in fictive swimming in zebrafish compared to active locomotion observed in earlier studies. Given that the CSF is a rich source of neuromodulators and endocrine signals, it could regulate the spontaneous firing of CSF-cNs via changes in pH or osmolarity via PKD2L1–14.8B or via ATP signalling at the P2X2 receptor. Tuning the spontaneous activity of CSF-cNs could modulate spontaneous locomotion in a similar manner as has recently been shown for sensory modulation of arousal locomotion in C. elegans. Altogether, our data demonstrate a novel role for CSF-cNs in the vertebrate spinal cord and opens new perspectives on a CSF sensory interface regulating locomotion.

**Methods**

**Animal care.** Animal handling and procedures were validated by the Institut du Cerveau et de la Moelle épinière, Paris and the French National Ethics Committee (Comité National de Réfexion Ethique sur l’Expérimentation Animale-Ces2011/ 056) in agreement with European Union legislation. Animals were reared at a maximal density of 8 animals per liter in a 14/10 (light/dark) cycle environment. Fish were fed live artemia twice a day and feeding regime was supplemented with a related publication. The swimming activity of embryos was measured during a 30 min swimming session in a stop chamber and the amount of amino acids released in the media was measured in the first transmembrane domain of Pkd2l1. All analysis was performed blind to genotype, which was only assayed at the conclusion of each experiment.

**Fluorescent immunohistochemistry.** Larvae were fixed in 4% PFA + 3% sucrose for 2 h at RT followed by 3-5 min washes in PBS. After removing the skin, larvae were embedded in 4% low melting agarose and cut in 50 μm sagittal sections. Sections were mounted on a slide and blocked for 1 h in blocking solution (10% NGS, 1% DMSO, 0.5% Triton X100 in 0.1 M PBS). Slides were incubated with primary antibody over night at RT or for two days at 4°C (1% NGS, 1% DMSO, 0.5% Triton X100 in 0.1 M PBS). After washing three times with 5 min in 0.1 M PBS with 0.5% TritonX100 (PBST), slides were incubated in the dark with the secondary antibody in PBST. After washing again three times for 3 min in PBST, mounting medium was added and slides were imaged on a confocal microscope using a 63X 1.4× Megachrome Plan Apochromat (Olympus). 

**Electron microscopy.** To label CSF-cNs for electron microscopy, we injected 60 ng/μl UAS:GFP-tagRFP into Tg(pkd2l1:Gal4;icm10) embryos at one-cell stage. Larvae were fixed in 0.125% glutaraldehyde/2% sucrose for 2 h on ice, embryos were rinsed with 0.125% glutaraldehyde/3% sucrose and then washed with 0.1 M sodium cacodylate buffer (pH 7.7–7.8). After fixation, embryos were dehydrated and infiltrated with 2×70% methanol, 3×20% methanol and 2×100% propylene oxide for 5 min each. The reaction was stopped by rinsing embryos in Na-cacodylate buffer post fixation in 2% osmium tetroxide and processed for standard electron microscopy. For sectioning, embryos were oriented in the coronal plane. Stained cells were identified in a wide-field microscope and regions of interest serially cut on copper grids to be further observed in a HITACHI 120 kV H7700 electron microscope.

**Calcium imaging setup.** 5–6 dpf larvae were anesthetized in 0.02% MS-222 diluted in fish facility water and then mounted in glass-bottom dishes (MatTek, Ashland, Massachusetts, USA) filled with 1.5% low melting point agarose. Calcium imaging was performed on a two photon microscope (2p-vivo, Intelligent Imaging Innovations, Inc., Denver) using a 20× objective. Images were acquired at 9 Hz (external pressure and paralyzed fish experiments) or 1 Hz (tail-free experiments). Experiments on paralyzed larvae: Larvae were paralyzed by injecting 0.5 μl of 10 mM hydrogen peroxide on the left side of the larva. The reaction was stopped by rinsing embryos in Na-cacodylate buffer post fixation in 2% osmium tetroxide and processed for standard electron microscopy. For sectioning, embryos were oriented in the coronal plane. Stained cells were identified in a wide-field microscope and regions of interest serially cut on copper grids to be further observed in a HITACHI 120 kV H7700 electron microscope.

**Generation of transgenic lines.** Transgenic lines used in this study are listed in Extended Data Table 1. We generated the Tg(pkd2l1:Gal4;icm10) transgenic line expressing the Gal4 reporter gene in pkd2l1 expressing cells, which amplified 3.8 kb of genomic sequence immediately upstream of the predicted ATG site for the zebrafish pzd2l1 gene (ENSDARG00000022503) as well as 630 bp of highly conserved DNA in intron 2. Both DNA fragments were then subcloned into the pZKhsph1 vector and converted to pH7.5 plasmid. Subcloning of the T2 promoter containing the pzd2l1 promoter, while the intron sequence was placed between the Gal4 stop codon and before the SV40 poly-A site. For generating the Tg(pkd2l1:GCaMP5G;icm07), the same strategy was used by regular cloning with the PT2 vector containing the GCaMP5G construct. Tg(UAS:GCaMP5G;icm08) was generated by regular cloning in the PT2–14xUAS vector. Tg(pkd2l1:Gal4;icm10) and Tg(UAS:GCaMP5G;icm08) were used for the following experiments. To generate a GCaMP cassette for TagRFP using restriction digest/ligation as above. To generate a GCaMP cassette for TagRFP using restriction digest/ligation as above. To generate a minimal promoter, while the intronic sequence was placed after the Gal4 (GFF) sequence in place of the SV40 poly-A site. Both DNA fragments were then subcloned into the pT2KhspGFF vector. The generation and validation of the UAS:BoTxLC-GFP line was based on the injection of Tg(pkd2l1:Gal4;icm10) embryos into Tg(pkd2l1:Gal4;icm10). Stable transgenic lines were established as previously described. The construct previously described was amplified by the reverse transcription kit (Invitrogen) and injected into Tg(pkd2l1:GCaMP5G;icm07) embryos at the one-cell stage. Injected embryos were pooled and a crude DNA extract was harvested. A DNA cassette containing the target site was amplified by PCR with primers: 5′-GAGGGCAAGAATGGACAAGACG-3′ and 5′-TGGTGGCTAACAGTGGGGG-3′, and then subcloned into the P2X2 vector to confirm disruption of the TALEN site. The mutant alleles were cloned by TOPO cloning (Invitrogen) and sequenced to determine the exact nature of the mutations. The Tg(pkd2l1:Gal4;icm10) mutant allele is an 8 bp deletion in exon 2 causing a 1 frameshift mutation. Tg(pkd2l1:Gal4;icm10) mutants were raised in a stop codon to prevent translation of the first transmembrane domain of Pkd2l1. All analysis was performed blind to genotype, which was only assayed at the conclusion of each experiment.

**Generation of pzd2l1 mutant.** RNA coding for each TALEN monomer (left arm target: 5′-TGAGAAAGATGGATACAAAAG-3′, right arm target: 5′-TTGAG ATTGTTGCTTCTATTCG-3′) was cloned into the pDONR221 and then assembled into the final construct previously described. This construct was used for microinjection at 1 frameshift mutation. Tg(pkd2l1:Gal4;icm10) mutants were raised in a stop codon to prevent translation of the first transmembrane domain of Pkd2l1. All analysis was performed blind to genotype, which was only assayed at the conclusion of each experiment.

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Calcium imaging data analysis. Regions of interest around CSF–CN cell bodies expressing both GCaMP and tagRFP were manually defined in the software ImageJ. To extract the fluorescence from the image time series in moving samples (either due to passive mechanical stimulation or active tail movement), the ROIs were shifted with the moving cell using a custom-made tracking algorithm based on cross-correlation. In frames where the normalized correlation coefficient fell underneath a given threshold due to the movement artifact, data were omitted for that given frame. This threshold was manually adjusted to minimize tracking errors for each imaging time series. Any ΔF/F values were calculated as ΔF/F = (F(t) – F₀)/(F₁ – F₀), with F₀ being the average fluorescence of the 10 first frames and F₁ the average fluorescence of the 10 frames in a region with only background signal. To correct for motion artifacts due to cells moving on the plane or incorrect tracking, we calculated the inverse of the GCaMP and the tagRFP signal (ΔR) as ΔR = (F₀CA×GCaMP(t) – F₀CA×GCaMP)/F₀GCaMP×GCaMP – F₀RFP×tagRFP) – 1 with F₀ being the average fluorescence of the 10 first frames and both F₀ and F(t) background corrected signals (see Fig. 3a and extended data Figure S2). Difference in ΔR due to stimulation was then calculated as difference = (ΔRpost,ΔRseq) with (ΔRpost,ΔRseq) being the average five time points before and after stimulation (external pressure experiments) or the average three time points before and after tail bending (free tail experiments). During motion of the tail, cells can usually not be imaged so both (ΔRpost,ΔRseq) and (ΔRpost,ΔRseq) there is a gap of 4 frames (external pressure experiment) or 1 frame (free tail experiment).

Behavior setup and analysis. To assess fish behavior, a video recording chamber was constructed. 6 dpf larvae from Tg(pkdl2iGcam520/2; -pdkl2l1:GCaMP5G) or Tg(pkdl2l1:GCaMP5G) were plated acutely in circular swim arenas (Biotech) atop a plexiglass plate. An Arduino Due software circuit (Arduino) was used to control the recording for high-speed videos of fish during escapes. The first executable, developed in collaboration with R&D Vision, fits a series of eleven points to the fish body—two for each of the eyes, one for the swim bladder, and the remaining eight to the tail. The program identifies these 11 points for each of the 650 frames of video. The second executable consists of a series of MATLAB scripts that derives kinetic parameters from these frames. First, the tail angle z was calculated by identifying the midpoint of the two eye points, then calculating a head direction vector based on this point and the center of the swim bladder. A tail direction vector based on the last point on the tail minus the swim bladder was then calculated, and z was calculated as the inverse cosine of these two vector lengths. To remove noise due to tracking errors, theraw trace of each angle (containing the average three time points before and after stimulation) was then fitted via linear mixed models for all our hypothesis testing. All models were calculated using the freeware R using the ‘nlme’ package. Detailed analysis scripts are available upon request.

Statistical analysis. All values are mean ± s.e.m. unless otherwise noted. To account for the fact that all our data are intrinsically nested (multiple measurements of cells and/or repetition of trials within the same fish), we used linear mixed models for all our hypothesis testing. All models were calculated using the freeware R using the ‘nlme’ package. Detailed analysis scripts are available upon request.

References

1. Martin, F. H., Seoane, J. R. & Baile, C. A. Feeding in satiated sheep elicited by intraventricular injections of CSF from fasted sheep. Life Sci. 13, 177–184 (1973).
2. Pappenheimer, J. R., Müller, T. B. & Goodrich, C. A. Sleep-promoting effects of cerebrospinal fluid from sleep-deprived goats. Proc. Natl Acad. Sci. USA 58, 513–517 (1967).
3. Lehtinen, M. K. et al. The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. J. Neurosci. 31, 909–911 (2011).
4. Sawamoto, K. et al. New neurons follow the flow of cerebrospinal fluid in the adult brain. Science 311, 629–632 (2006).
5. Xie, L. et al. Sleep drives metabolite clearance from the adult brain. Science 342, 373–377 (2013).
6. Agdher, E. Über ein zentrales Sinnesorgan (I) bei den Vertebraten. Z. Anat. Entwickelt. Gesch. 66, 233–312 (1922).
7. 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).
8. Orts-DeImmagino, A. et al. Morphology, distribution and phenotype of polysynaptic kidney disease 2-like 1-positive cerebrospinal fluid-contacting neurons in the brainstem of adult mice. PLoS ONE 9, e87744 (2014).
9. Vogl, B. & Vogl-Teichmann, I. Actual problems of the cerebrospinal fluid-contacting neurons. Microsc. Res. Tech. 41, 57–98 (1998).
10. Kolmer, W. Das ‘Sagitallorgan’ der Wirbeltiere. Z. Anat. EntwGesch. 60, 652–717 (1921).
11. Dale, N., Roberts, A., Ottersen, O. P. & Mathisen, J. S. 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. 223, 193–203 (1987).
12. Wuyt, C. et al. Optogenetic dissection of a behavioural module in the vertebrate spinal cord. Nature 461, 407–410 (2009).
13. Fidelin, K. et al. State-dependent modulation of locomotion by GABAergic spinal sensory neurons in vivo, connection-specific. J. Physiol. (Lond.) 513, 5035–5047 (2015).
14. Rushman, J. D., Ye, W. & Liman, E. R. A proton current associated with sour taste: Distribution and functional properties. FASEB J. 29, 3014–3026 (2015).
15. Huang, H. A. et al. The cells and logic for mammalian sour taste detection. Nature 442, 934–938 (2006).
16. Orts DeImmagino, A. et al. Properties of subependymal cerebrospinal fluid contacting neurons in the dorsal vagal complex of the mouse brainstem. J. Physiol. (Lond.) 590, 3719–3741 (2012).
17. Stoeckel, M.-E. et al. Cerebrospinal fluid-contacting neurons in the rat spinal cord, a ?-aminobutyric acidergic system expressing the P2X2 subunit of purinergic receptors, PSA-NCGAM, and GAP-43 immunoreactivities: Light and electron microscopic study. J. Comp. Neurol. 457, 159–174 (2003).
18. Kolmer, W. Über das Sagittalorgan, ein zentrales Sinnesorgan der Wirbeltiere, insbesondere beim Affen. Z. Zellforsch. 13, 236–248 (1931).
19. Masino, M. A. & Fetto, J. R. Fictive swimming motor patterns in wild type and mutant larval zebrafish. J. Neurophysiol. 93, 3177–3188 (2005).
20. McLean, D. L., Fan, J., Higashijima, S.-I., Hale, M. E. & Fetcho, J. R. A hyperpolarizing map of recruitment in spinal cord. Nature 446, 71–75 (2007).
21. Schredllesker, J. et al. The beta 1a subunit is essential for the assembly of dihydropyridine-receptor arrays in skeletal muscle. Proc. Natl Acad. Sci. USA 102, 17219–17224 (2005).
22. Sternberg, J. R. et al. Optimization of a neurotoxin to investigate the contribution of excitatory interneurons to speed modulation in vivo (in preparation).
23. Delling, M., DeCaen, P. G., Doerner, J. F., Fenvay, S. & Clapham, D. E. Primary cilia are specialized calcium signalling organelles. Nature 504, 311–314 (2013).
24. Hu, M., Liu, Y., Wu, J. & Liu, X. Influx-operated Ca\(^{2+}\) entry via PKD2-L1 and PKD1-L3 channels facilitates sensory responses to polynuclear transient stimuli. *Cell Reports* **13**, 798–811 (2015).

25. Chang, R. B., Waters, H. & Liman, E. R. A proton current drives action potentials in genetically identified sour taste cells. *Proc. Natl Acad. Sci. USA* **107**, 22320–22325 (2010).

26. Orts-De'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 (2015).

27. Jalalvand, E., Robertson, B., Wallen, P. & Grillner, S. Ciliated neurons lining the central canal sense both fluid movement and pH through ASIC3. *Nat. Commun.* **7**, 10002 (2016).

28. Grillner, S., McClellan, A. & Perret, C. Entrainment of the spinal pattern generators for swimming by mecano-sensitive elements in the lamprey spinal cord in vitro. *Brain Res.* **217**, 380–386 (1981).

29. Borgmann, A., Hooper, S. L. & Büschges, A. Sensory feedback induced by front-leg stepping entrains the activity of central pattern generators in caudal segments of the stick insect walking system. *J. Neurosci.* **29**, 2972–2983 (2009).

30. Pearson, K. G. Role of sensory feedback in the control of stance duration in walking cats. *Brain Res. Rev.* **57**, 222–227 (2008).

31. Wallen, P. & Williams, T. L. Fictive locomotion in the lamprey spinal cord in vitro compared with swimming in the intact and spinal animal. *J. Physiol. (Lond.)* **347**, 225–239 (1984).

32. Sqalli-Houssaini, Y., Cazalets, J. R., Fabre, J. C. & Clarac, F. A cooling/heating system for use with in vitro preparations: study of temperature effects on newborn rat rhythmic activities. *J. Neurosci. Methods* **39**, 131–139 (1991).

33. Chou, S. et al. Sensory neurons arouse *C. elegans* locomotion via both glutamate and neuropeptide release. *PLoS Genet.* **11**, e1005359 (2015).

34. Chen, T.-W. et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**, 295–300 (2013).

35. Zelenchuk, T. A. & Brusé, J. L. In Vivo labeling of zebrafish motor neurons using an mnx1 enhancer and Gal4/UAS. *Genesis* **49**, 546–554 (2011).

36. Fisher, S. et al. Evaluating the biological relevance of putative enhancers using Tol2 transposon-mediated transgenesis in zebrafish. *Nat. Protoc.* **1**, 1297–1305 (2006).

37. Lam, S. S. et al. Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nat. Meth.* **12**, 51–54 (2015).

38. Saint-Amant, L. & Drapeau, P. Whole-cell patch-clamp recordings from identified spinal neurons in the zebrafish embryo. *Methods Cell Sci.* **25**, 59–64 (2003).

39. Joselevitch, C. & Zenisek, D. Imaging exocytosis in retinal bipolar cells with TIRF microscopy. *J. Vis. Exp.* **28**, doi:103791/1305 (2009).

40. Aarts, E., Verhage, M., Veenwijl, J. V., Dolan, C. V. & van der Sluis, S. A solution to dependency: using multilevel analysis to accommodate nested data. *Nat. Neurosci.* **17**, 491–496 (2014).

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**Author contributions**

U.B. performed calcium imaging and electrophysiology experiments. A.P. and S.K. performed behavioural experiments. A.P. and L.D. generated the mutants with help of M.C. and J.P.C. with TALENs. S.N.F. performed IHC, cultures, recorded beating cilia and contributed to EM data. J.P.R. acquired the EM data with the help of L.D. and J.G. C.S. recorded cilia beating in vivo. A.P., S.N.F., M.S., K.K. and F.D.B. generated transgenic animals. U.B., A.P. and C.W. designed experiments, analyzed data and wrote the manuscript.

**Additional information**

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