

Ca²⁺-activated Cl⁻ current ensures robust and reliable signal amplification in vertebrate olfactory receptor neurons

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Edited by John E. Dowling, Harvard University, Cambridge, MA, and approved November 28, 2018 (received for review September 21, 2018)

Activation of most primary sensory neurons results in transduction currents that are carried by cations. One notable exception is the vertebrate olfactory receptor neuron (ORN), where the transduction current is carried largely by the anion Cl⁻. However, it remains unclear why ORNs use an anionic current for signal amplification. We have sought to provide clarification on this topic by studying the so far neglected dynamics of Na⁺, Ca²⁺, K⁺, and Cl⁻ in the small space of olfactory cilia during an odorant response. Using computational modeling and simulations we compared the outcomes of signal amplification based on either Cl⁻ or Na⁺ currents. We found that amplification produced by Na⁺ influx instead of a Cl⁻ efflux is problematic for several reasons: First, the Na⁺ current amplitude varies greatly, depending on mucosal ion concentration changes. Second, a Na⁺ current leads to a large increase in the ciliary Na⁺ concentration during an odorant response. This increase inhibits and even reverses Ca²⁺ clearance by Na⁺/Ca²⁺/K⁺ exchange, which is essential for response termination. Finally, a Na⁺ current increases the ciliary osmotic pressure, which could cause swelling to damage the cilia. By contrast, a transduction pathway based on Cl⁻ efflux circumvents these problems and renders the odorant response robust and reliable.

olfaction | Ca-activated Cl current | mathematical modeling | electrodiffusion | cilia ion dynamics

Olfactory receptor neurons (ORNs) in the nasal olfactory epithelium are the fundamental neurons underlying the sense of smell (1). ORNs are bipolar neurons that extend an axon to the olfactory bulb and a single dendrite to the epithelial border, ending in the dendritic knob (Fig. 1*A*). The cellular compartments that detect and transduce odorants into an electrical signal are the olfactory cilia. There are about 10–15 long and slender cilia per ORN embedded in the mucus on the surface of the olfactory epithelium. The ciliary membrane contains odorant receptors that are activated by odorant molecules carried into the nose during inhalation and dissolved in the mucus. Receptor activation initiates a biochemical transduction cascade that depolarizes the neuron via the opening of ion channels located in the ciliary membrane (Fig. 1*B*).

Unlike in other sensory systems like taste, hearing, or phototransduction (2), a remarkable feature of olfactory signal transduction is that it involves not only the opening of a cation channel but also that of an anion channel (Fig. 1*B*), which jointly carry all of the odorant-induced current. Receptor activation first leads, via a G protein, to activation of adenylyl cyclase 3 (AC3) that synthesizes cAMP. The subsequent increase in the ciliary cAMP concentration opens cAMP-gated, cyclic nucleotide-gated (CNG) cation channels that are permeable to Ca²⁺ and to a lesser extent to Na⁺ and K⁺ (3, 4). The Ca²⁺ influx triggers the opening of a secondary anionic channel: the Ca²⁺-gated Cl⁻ channel [Anoctamin 2 (Ano2), also known as TMEM16B] (5–8). Cl⁻ efflux ensues, as intracellular Cl⁻ is high such that the

Nernst equilibrium potential for Cl⁻ is positive to the ORN resting potential (9, 10). Ciliary Ca²⁺ is predominantly removed by electrogenic Na⁺/K⁺/Ca²⁺ exchange (NCKX4) (11). The activation of the Ano2 channels is the main amplification step, and most of the transduction current is carried by Cl⁻ efflux (12–16). This large amplification is thought to be the principal function of the Cl⁻ current. When the Cl⁻ current is deleted either pharmacologically or by deletion of the Ano2 gene, mice retain some sense of smell due to the CNG channels, but ORN responses are much smaller, affecting sensitivity at the signaling threshold, the ability to track novel odorants, and spiking activity (13, 17–19).

It is still unclear why ORNs rely upon a Cl⁻ current to boost the amplitude of their response (21, 22). A larger signal could be produced if ORNs had more CNG channels, but a simple increase in channel number would produce larger influx of both Na⁺ and Ca²⁺, which would disturb Ca²⁺ homeostasis and affect several Ca²⁺-dependent feedback processes in the biochemical transduction pathway (1, 23). We note that a cilium has a volume of only about 0.5 fL, and a current of just 1 pA already produces an ion flux of about 20 mM/s. Thus, with a large CNG current one expects a strong increase in intracellular Na⁺ and Ca²⁺ concentrations with currently unclear consequences. For example, robustness of Ca²⁺ clearance could be affected because the intracellular Ca²⁺ concentration would increase due to larger

Significance

The cilia of olfactory receptor neurons, embedded in the nasal mucus, are tiny organelles that detect and transduce inhaled odorants into electrical currents. We addressed the long-standing question of why the olfactory transduction pathway relies on the activation of a depolarizing Cl⁻ current as the main amplification step, contrary to an otherwise more common Na⁺ current. Our results show that a Cl⁻ as opposed to a Na⁺ current provides several major advantages: It renders the odorant response robust against mucosal ion concentration changes, it keeps the intracellular Na⁺ concentration low to ensure Ca²⁺ clearance, and it prevents an increase in the ciliary osmotic pressure. As a result, olfaction functions faithfully even in an unstable nasal environment with fluctuating ion concentrations.

Author contributions: J. Reisert and J. Reingruber designed research; J. Reisert performed electrophysiological recordings; J. Reingruber implemented the model; and J. Reingruber and J. Reisert wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1816371116/-DCSupplemental.

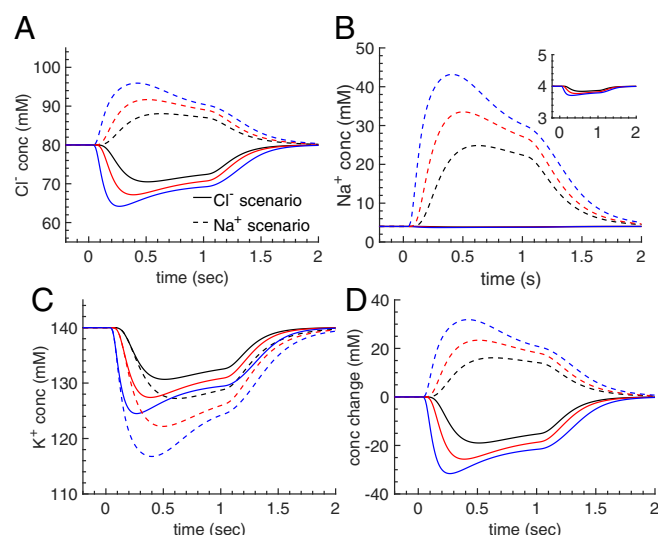


Fig. 3. Spatially averaged ion concentrations in a cilium. Solid lines correspond to the Cl^- and dashed lines to the Na^+ scenario. (A–C) Comparison of the dynamics for Cl^- , Na^+ , and K^+ concentrations corresponding to the odorant responses from Fig. 2. *B, Inset* details the Na^+ concentration for the Cl^- scenario. (D) Osmotic concentration change.

for both scenarios are performed with exactly the same set of parameters except for the two parameters ν_{ano}^{cl} and ν_{ano}^{na} that determine the Ano2 permeability to Cl^- and Na^+ (*SI Appendix, Table S1*). In the Cl^- scenario we have ($\nu_{ano}^{cl} = 7.6 \text{ s}^{-1}$, $\nu_{ano}^{na} = 0$), and in the Na^+ scenario we have ($\nu_{ano}^{cl} = 0$, $\nu_{ano}^{na} = 3.4 \text{ s}^{-1}$). The smaller Na^+ permeability adjusts for the larger Na^+ electrochemical driving force between cilia and mucus such that an odorant stimulation induces similar currents in both scenarios.

In Fig. 2 *C–F*, we compare results that are spatially averaged along the length of a cilium for quantities that are commonly used in standard olfactory models: cAMP and Ca^{2+} concentration and ciliary and cell body depolarization. Fig. 2 does not suggest any advantage for Cl^- as current carrier since simulation results are very similar in the two scenarios. More generally, with a standard cable theory model where ionic concentration changes and their effects are neglected (except for Ca^{2+}), it makes no difference for the electrical response whether the current is carried by Cl^- or Na^+ . With such a model the simulations in Fig. 2 for both scenarios would exactly match.

The spatially averaged results for the Na^+ , Cl^- , and K^+ concentrations, on the other hand, reveal large differences between the two scenarios (Fig. 3, solid vs. dashed lines). In the Cl^- scenario, the ciliary Cl^- concentration drops due to Cl^- efflux into the mucus (Fig. 3A, solid lines). In strong contrast, the Na^+ concentration changes very little (Fig. 3B, solid lines and *Inset*). Note that we neglect Na^+ influx through CNG channels, which would lead to a small increase in the ciliary Na^+ concentration instead of the slight decrease seen in Fig. 3B. The K^+ concentration decreases due to efflux into the cell body driven by the ciliary depolarization (Fig. 3C, solid lines). Na^+ is less affected because the internal Na^+ concentration is much lower (4 mM). In the Cl^- scenario the declines of K^+ and Cl^- electrically compensate one another such that electroneutrality is preserved. The situation changes with a Na^+ current. With Na^+ influx the ciliary Na^+ concentration is dramatically increased by up to 40 mM (Fig. 3B, dashed lines). The ciliary depolarization causes the Cl^- concentration to increase (Fig. 3A, dashed lines) and the K^+ concentration to decrease (Fig. 3C, dashed lines) via exchanges with the cell body. The changes in the K^+ and Cl^- concentrations no longer electrically compensate as in the Cl^- scenario, but

instead they generate a large gap of positive charge that is filled with Na^+ flowing in from the mucus to maintain electroneutrality. Finally, we note that the overall ion concentration in a cilium drops with a Cl^- current, whereas it increases with a Na^+ current (Fig. 3D). Hence, osmotic concentration and pressure change in opposite ways in the two scenarios. In summary, we found complex and highly coupled ion dynamics in the cilia due to the small ciliary volume and the requirement for electroneutrality. In [SI Appendix, Fig. S2](#) we show that modifying only the K^+ dynamics also affects the coupled Cl^- and Na^+ concentrations. With a 10-fold larger ciliary diameter (similar to a rod photoreceptor outer segment), intracellular concentration changes become small due to the large volume, and an effective model with constant ion concentrations would now be justified ([SI Appendix, Fig. S3](#)).

Large Ionic Concentration Gradients Are Generated in a Cilium During an Odorant Response. Next, we investigated the degree of spatial inhomogeneity that is generated in a cilium during an odorant response. In Fig. 4 we compare spatial distributions for ion concentrations, Ca^{2+} efflux via the exchangers, and ciliary voltage for the strongest odorant stimulation in Fig. 2 at four different times: before, twice during, and once after the 1-s odorant stimulus. Solid lines correspond to the Cl^- scenario and dashed lines to the Na^+ scenario. In agreement with Fig. 3, we find large differences between the two scenarios for the Cl^- and Na^+ concentrations (Fig. 4*A* and *B*) and to a lesser extent for the K^+ concentration (Fig. 4*C*). With Cl^- as the current carrier, almost

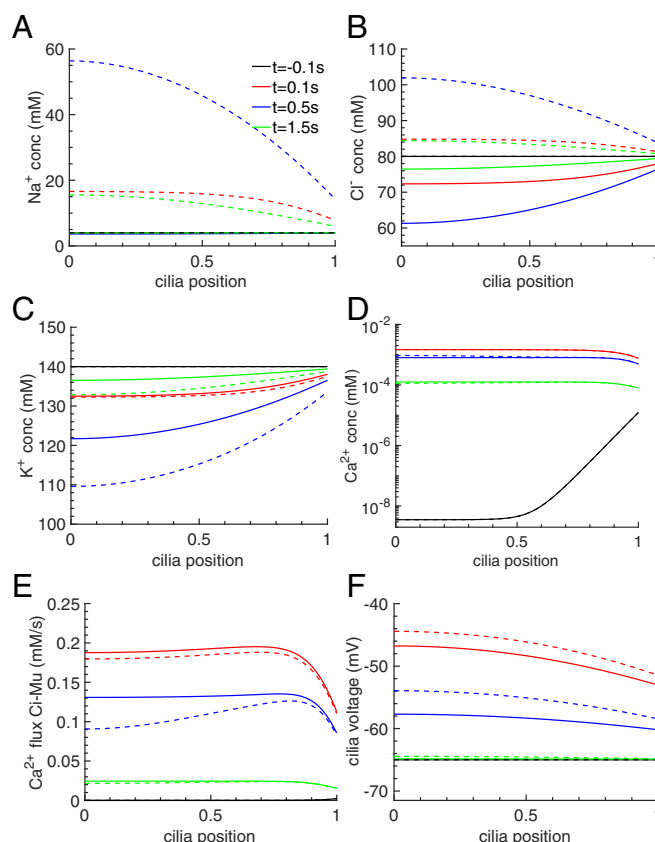


Fig. 4. Spatial distributions along a cilium at four different times. The rescaled ciliary position is between zero and one. Zero marks the tip and one the base of a cilium. (A–D) Na^+ , Cl^- , K^+ , and Ca^{2+} gradients, respectively. (E and F) Ca^{2+} efflux due to NCKX4 exchange (E) and the ciliary voltage (F). Solid lines show results from the Cl^- scenario and dashed lines those from the Na^+ scenario. The distributions correspond to Figs. 2 and 3 for the strongest odorant stimulation (100 μM).

With a Na^+ Current the Odorant Response Is Not Robust Against Ionic Concentration Changes in the Mucus. Next, we addressed the important question of whether the odorant response is robust against mucosal ion concentration changes. To test robustness, we performed simulations for the highest odorant stimulation with the same parameters as before, but we reduced the mucosal concentrations of Na^+ and Cl^- from 140 mM to 100 mM and 70 mM each (Fig. 5 *A* and *B*). Such values are not unrealistic as several reports suggest that mucosal concentrations can be significantly lower than the 140 mM in Ringer solution used in electrophysiological experiments (27–29). With a Cl^- current, the response is little affected by changing mucosal concentrations (Fig. 5*A*). For example, the peak amplitude of around 240 pA for 70 mM is only slightly increased compared with around 210 pA for 140 mM due to the larger driving force. In contrast, with a Na^+ current, the response is strongly altered (Fig. 5*B*). The initial peak amplitude for 70 mM is almost halved compared with that for 140 mM. In brief (for a more detailed analysis we refer to *SI Appendix, Eqs. S9 and S10*), Cl^- efflux is determined by the ciliary Cl^- concentration; altering the mucosal concentration affects this result only a little. In contrast, Na^+ influx is proportional to the mucosal Na^+ concentration and is strongly affected by mucosal concentration changes. This result shows

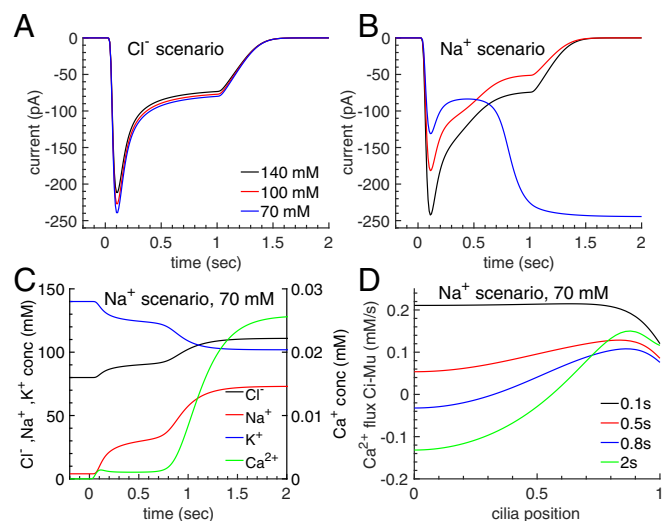


Fig. 5. Stability of the odorant response in a varying mucosal environment. (A and B) Comparison of the current for strongest odorant stimulation and mucosal concentrations of Cl^- and Na^+ of 140 mM, 100 mM, and 70 mM. (A) Cl^- scenario. (B) Na^+ scenario. (C and D) Results for the Na^+ scenario with 70 mM. (C) Spatially averaged ion concentrations. (D) Ca^{2+} flux between cilium and mucus generated by NCKX4 exchange. A positive flux corresponds to efflux from a cilium.

that a Cl^- but not a Na^+ current is robust against mucosal ion concentration changes.

Finally, a very surprising effect occurs in the Na^+ scenario due to our artificial Na^+ channels that are Ca^{2+} activated. When the mucosal concentrations are reduced to 70 mM, at a time around 0.7 s, instead of declining, the current increases again and reaches a plateau value that persists even after the odorant stimulation ends at 1 s (Fig. 5*B*, blue line). Correspondingly, intracellular ion concentrations attain plateau values that are very different from their initial values (Fig. 5*C*). The system is obviously bistable with two stable fixed points in the absence of odorant stimulation: one with zero current and one with a large current. The reason for this bistability is a positive feedback loop that is present if the Na^+ channels are Ca^{2+} activated: A sufficiently large Na^+ current increases the ciliary Na^+ concentration such that the exchangers eventually switch to reverse mode in the frontal part of the cilium (Fig. 5*D*, negative current near the tip). In reverse mode, the exchangers import Ca^{2+} from the mucus, which keeps the Ca^{2+} -activated Na^+ channels open even without odorant stimulation. In Fig. 5*B*, the odorant stimulation pushes the system into the basin of attraction of the nonzero fixed point, which abolishes response termination. In contrast, bistability does not occur with a Ca^{2+} -activated Cl^- current because the ciliary Na^+ concentration remains low and exchangers do not switch to reverse mode.

Discussion

We studied the ciliary ion dynamics during an odorant response to elucidate a long-standing question in olfactory transduction: Why is amplification in olfactory transduction carried by a Cl^- and not a Na^+ current? To answer this question, we developed a mathematical model that predicts the spatiotemporal ion dynamics in a cilium. Compared with previous models that focus on the biochemical signal transduction pathway and on the Ca^{2+} dynamics (30–33), we consider the coupled dynamics between Cl^- , Na^+ , K^+ , and Ca^{2+} ions in the constrained volume of a cilium during an odorant response. Moreover, we did not rely on an effective equation for Ca^{2+} extrusion that depends only on the ciliary Ca^{2+} concentration, but we considered that the NCKX4

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Supporting Information Appendix

Johannes Reisert and Jürgen Reingruber

1 Derivation of the spatially resolved model

The initial steps in the signal transduction pathway occur in long and slender cilia, for reviews see [1, 2, 3]. The activation of an odorant receptor in the ciliary membrane initiates a G protein-coupled cascade that leads to the opening of CNG channels. The resulting Ca^{2+} influx activates Ca^{2+} -activated Cl^- channels (Ano2) that further amplify the response. Ciliary cAMP is degraded by phosphodiesterases and Ca^{2+} is removed by $Na^+/K^+/Ca^{2+}$ exchangers (NCKX4) [4]. This leads to closure of CNG and Ano2 channels and response termination.

1.1 Electrodiffusion model for the ion dynamics in a cilium

To study the ion dynamics in a cilium, we use an electrodiffusion model similarly to the models in [5, 6, 7]. We model a cilium as a rotationally symmetric cylinder of length L_{ci} and radius R_{ci} with membrane surface $S_{ci} = 2\pi R_{ci} L_{ci}$, cross section $A_{ci} = \pi R_{ci}^2$ and cytoplasmic volume $V_{ci} = A_{ci} L_{ci}$. We neglect the ciliary space occupied by axoneme since it would not qualitatively change our results. We assume that a cilium is rotationally symmetric and radially homogeneous and we introduce the dimensionless position $z = x/L_{ci}$. Because $R_{ci} \ll L_{ci}$, we assume that a cilium is radially homogeneous. Hence, ciliary quantities depend only on the time and the longitudinal position $0 \leq x \leq L_{ci}$. Instead of x we use the dimensionless position $z = x/L_{ci}$. The cilium tip is at $z = 0$ and the cilium base at $z = 1$ is the connection to the dendritic knob. We further introduce the dimensionless voltage $\phi = \frac{U}{U_T}$, where $U_T = \frac{RT}{\mathcal{F}} \approx 25mV$ at $T = 293K$ ($\mathcal{F}$ is the Faraday constant and R the ideal gas constant). We consider the dynamics of K^+ , Na^+ , Ca^{2+} and Cl^- . We assume that negatively charged organic anions are immobile. As a consequence, no currents are associated with them and they do not contribute to the dynamic. However, it is straightforward to include their dynamics in a more detailed version of our model.

Ions of valence z_s diffuse with diffusion constant D_s , where $s = ca, cl, na, k$ labels the ion species. The ciliary concentrations $c_{ci}^s(z, t)$ change due to membrane fluxes $J_{ci, mu}^s(z, t)$ and longitudinal fluxes $J_{ci}^s(z, t)$. Fluxes depend on channel and exchanger properties, and concentration and voltage gradients. We introduce the modified fluxes

$$\begin{aligned} j_{ci, mu}^s &= \frac{dS_{ci}}{dV_{ci}} J_{ci, mu}^s = \frac{2}{R_{ci}} J_{ci, mu}^s \\ j_{ci}^s &= \frac{J_{ci}^s}{L_{ci}} = -\nu_{ci}^s (\partial_z c_{ci}^s + z_s c_{ci}^s \partial_z \phi_{ci}) , \end{aligned} \tag{1}$$

where $\nu_{ci}^s = \frac{D_s}{L_{ci}^2}$ are diffusional rate constants. Note that $j_{ci, mu}^s$ and j_{ci}^s have unit $\frac{mM}{s}$. The flux through a surface element dS_{ci} is $J_{ci, mu}^s dS_{ci} = j_{ci, mu}^s dV_{ci} = V_{ci} j_{ci, mu}^s dz$, and the longitudinal flux is

$A_{ci}J_{ci}^s = V_{ci}j_{ci}^s$. The modified fluxes have intuitive interpretations: during one second, a uniform flux would change the ciliary concentration by j . The currents associated with the fluxes are

$$\begin{aligned} I_{ci,mu}^s &= z_s \mathcal{F} V_{ci} j_{ci,mu}^s \\ I_{ci}^s &= z_s \mathcal{F} V_{ci} j_{ci}^s. \end{aligned} \quad (2)$$

With $V_{ci} \sim 0.5 \mu\text{m}^3$ we find $\mathcal{F} V_{ci} \sim 0.05 \frac{\text{s pA}}{\text{mM}}$. Thus, a current of 1pA corresponds to a flux of $1/0.05=20$ mM/s. Hence, in one millisecond a current of 100pA can, in principle, change the ciliary concentration by 2 mM. This illustrates that currents can quickly alter the ciliary concentrations.

We apply the usual convention that inward fluxes are negative and outward fluxes are positive. Thus, an inward flux of cations or an outward flux of anions generate a negative current. The conservation equations (Nernst-Planck electrodiffusion equations) for the ion concentrations are [8]

$$\partial_t c_{ci}^s = -\partial_z j_{ci}^s - j_{ci,mu}^s. \quad (3)$$

We ignore slow Ca^{2+} buffers and we use the instantaneous buffer approximation with an effective slow Ca^{2+} diffusion constant due to rapid bindings and unbindings. Due to the large mucosal volume, we neglect concentration and potential changes in the mucus during an odorant response. However, we do explore the impact of different values for the mucus concentrations. We use the mucus potential as reference and we set $\phi_{mu} = 0$ such that $\phi_{ci} - \phi_{mu} = \phi_{ci}$.

A challenge is to compute the spatio-temporal dynamics of the ciliary potential $\phi_{ci}(z, t)$. The most accurate way would be to solve the Poisson equation with appropriate boundary conditions, leading to the Poisson-Nernst-Planck (PNP) coupled system of equations. However, such a complex system of partial differential equations, with additional feedback from the cell body dynamics, would be very demanding for numerical simulations. In particular, we are interested in simulations that lasts for seconds and not only milliseconds or steady state results [9, 10, 11]. To reduce complexity, we use the charge-capacitor approximation to compute the potential as a function of the electric currents and the membrane capacitance [5, 12, 7]

$$\partial_t \phi_{ci} = \frac{1}{C_{ci} U_T} \sum_s (-\partial_z I_{ci}^s - I_{ci,mu}^s). \quad (4)$$

$C_{ci} = S_{ci} C_m$ is the cilium capacitance ($C_m = 1 \mu\text{F}/\text{cm}^2 = 10^{-5} \text{nF}/\mu\text{m}^2$ is the membrane capacity). Although Eq. 4 is formally identical to the cable equation [13], we note that ionic concentrations are not constant in Eq. 4, as it is the case for the cable equation.

1.2 Well-stirred model for the cell body

Due to the large size of the cell body, we approximate the cell body concentrations c_{cb}^s as being constant. It is straightforward to relax this assumption in a more sophisticated future model. Because of many uncertainties concerning properties and distribution of voltage gated channels in the cell body, and because it is of little relevance for the ciliary ion dynamics, we do not include a mechanism for action potential generation into our cell body model. Instead, we adopt the simplest possible model where the cell body potential $\phi_{cb}(t)$ changes due to a leak current, which defines the resting potential, and the currents from N_{ci} identical cilia,

$$\frac{\partial}{\partial t} \phi_{cb} = \frac{N_{ci}}{C_{cb} U_T} \sum_s I_{ci,cb}^s - \frac{g_{cb}^{leak}}{C_{cb}} (\phi_{cb} - \phi_{cb}^{leak}). \quad (5)$$

C_{cb} is the cell body capacitance, g_{cb}^{leak} is the leak conductance, ϕ_{cb}^{leak} is the leak reversal potential and $I_{ci,cb}^s(t) = I_{ci}^s(1, t) = z_s \mathcal{F} V_{ci} j_{ci}^s(1, t)$ is the current from a cilium carried by ion species s .

1.3 Ion fluxes between cilium and mucus

The fluxes between cilium and mucus depend on CNG and Ano2 channels and electrogenic NCKX4 exchangers

$$j_{ci,mu}^s = j_{ci,mu}^{s,cng} + j_{ci,mu}^{s,ano} + \zeta_{nckx}^s j_{ci,mu}^{nckx}. \quad (6)$$

The numbers ζ_{nckx}^s define the exchange stoichiometry.

1.3.1 Fluxes via CNG and Ano2 channels

Similar to [14, 15], we use the Goldman-Hodgkin-Katz (GHK) flux equation [13] to compute channel fluxes between cilia and mucus. With $\phi_{mu} = 0$ such that $\phi_{ci,mu} = \phi_{ci} - \phi_{mu} = \phi_{ci}$ and the approximation

$$\frac{x}{e^{x/2} - e^{-x/2}} \approx e^{-\frac{x^2}{24}} \quad (7)$$

valid for $|x| \lesssim 5$ we have

$$j_{ci,mu}^{s,ch} = p_{ch} \nu_{ch}^s e^{-\frac{(z_s \phi_{ci})^2}{24}} \left(c_{ci}^s e^{z_s \phi_{ci}/2} - c_{mu}^s e^{-z_s \phi_{ci}/2} \right) \quad (8)$$

$$= p_{ch} \nu_{ch}^s e^{-\frac{(z_s \phi_{ci})^2}{24}} c_{ci}^s e^{z_s \phi_{ci}/2} \left(1 - \frac{c_{mu}^s}{c_{ci}^s} e^{-z_s \phi_{ci}} \right) \quad (9)$$

$$= -p_{ch} \nu_{ch}^s e^{-\frac{(z_s \phi_{ci})^2}{24}} c_{mu}^s e^{-z_s \phi_{ci}/2} \left(1 - \frac{c_{ci}^s}{c_{mu}^s} e^{z_s \phi_{ci}} \right). \quad (10)$$

$p_{ch}(z, t)$ is the channel open probability and the rate $\nu_{ch}^s \sim \frac{\rho_{ch}^s \gamma_{ch}^s}{R_{ci}}$ is proportional to the channel density ρ_{ch} and the single channel conductivity γ_{ch}^s and R_{ci}^{-1} (due to Eq. 1).

Eq. 9 shows that Cl^- efflux is proportional to the ciliary Cl^- concentration, and altering the mucosal Cl^- concentration affects this efflux only by little via the term $1 - c_{mu}^d/c_{ci}^d e^{\phi_{ci}}$. In contrast, Eq. 10 shows that Na^+ influx is proportional to the mucosal Na^+ concentration and is therefore strongly affected by mucosal concentration changes (we have $1 - c_{ci}^{na}/c_{mu}^{na} e^{\phi_{ci}} \approx 1$).

The open probability for Ano2 channels $p_{ano}(z, t)$ depends on the Ca^{2+} concentration [16, 17],

$$p_{ano} = \frac{(c_{ci}^{ca})^{h_{ano}}}{(c_{ci}^{ca})^{h_{ano}} + K_{ano}^{h_{ano}}}, \quad (11)$$

with $K_{ano}^{ca} \approx 1.8 \mu M$ and $h_{ano} \approx 2.3$ [17]. The CNG open probability $p_{cng}(z, t)$ depends on the cAMP concentration $c_{camp}(z, t)$ [18, 19]. Furthermore, CNG channels become desensitized due to negative Ca^{2+} feedback via calmodulin (CaM) [20, 21]. In a first approximation, we incorporate this negative feedback using a Ca^{2+} -dependent Hill concentration $K_{cng}(z, t)$,

$$p_{cng} = \frac{(c_{ci}^{camp})^{h_{cng}}}{(c_{ci}^{camp})^{h_{cng}} + K_{cng}^{h_{cng}}} \quad (12)$$

with

$$K_{cng} = K_{cng}^{min} \left(1 + f_{cng}^{ca} \frac{c_{ci}^{ca}}{c_{ci}^{ca} + K_{cng}^{ca}} \right). \quad (13)$$

1.3.2 Fluxes via NCKX4 exchangers

During one cycle, 4 Na^+ are exchanged for one K^+ and one Ca^{2+} ($\zeta_{nckx}^{na} = -4$, $\zeta_{nckx}^{ca} = \zeta_{nckx}^k = 1$). Hence, NCKX4 is an electrogenic exchanger and the net current is

$$I_{ci,mu}^{nckx} = \mathcal{F}V_{ci} \sum_s z_s \zeta_{nckx}^s j_{ci,mu}^{nckx} = r \mathcal{F}V_{ci} j_{ci,mu}^{nckx}, \quad (14)$$

where $r = \sum_s z_s \zeta_{nckx}^s = -1$. The flux $j_{ci,mu}^{nckx}$ corresponds to either Ca^{2+} or K^+ . To obtain a formula for $j_{ci,mu}^{nckx}(z, t)$ we generalize the expression for the NCX exchanger [14, 22, 23],

$$\begin{aligned} j_{ci,mu}^{nckx} &= \nu_{nckx} \frac{c_{ci}^{ca} (c_{mu}^{na})^4 c_{ci}^k e^{r\phi_{ci}/2} - c_{mu}^{ca} (c_{ci}^{na})^4 c_{mu}^k e^{-r\phi_{ci}/2}}{(c_{ci}^{ca} + K_{nckx})(c_{mu}^{na})^4 c_{ci}^k + (c_{mu}^{ca} + K_{nckx})(c_{ci}^{na})^4 c_{mu}^k} \\ &= \nu_{nckx} c_{ci}^{ca} (c_{mu}^{na})^4 c_{ci}^k e^{r\phi_{ci}/2} \frac{1 - e^{-r\phi_{ci} + \sum_s z_s \zeta_{nckx}^s \phi_{ci,mu}^s}}{(c_{ci}^{ca} + K_{nckx})(c_{mu}^{na})^4 c_{ci}^k + (c_{mu}^{ca} + K_{nckx})(c_{ci}^{na})^4 c_{mu}^k} \end{aligned} \quad (15)$$

with the Nernst potentials

$$\phi_{ci,mu}^s = \frac{1}{z_s} \ln \left(\frac{c_{mu}^s}{c_{ci}^s} \right). \quad (16)$$

$j_{ci,mu}^{nckx}$ depends symmetrically on ciliary and mucosal parameters. The flux vanishes when the free energy change is zero, $\Delta E \sim \sum_s z_s \zeta_{nckx}^s (\phi_{ci,mu}^s - \phi_{ci}) = 0$. The ratio between ciliary and mucus Ca^{2+} concentration at this point is

$$\frac{c_{ci}^{ca}}{c_{mu}^{ca}} = \left(\frac{c_{ci}^{na}}{c_{mu}^{na}} \right)^4 \frac{c_{mu}^k}{c_{ci}^k} e^{-r\phi_{ci}}. \quad (17)$$

At rest, with $\phi_{ci} = -2.4$ (corresponding to -65mV), $c_{mu}^{na} = 140mM$, $c_{ci}^{na} = 4mM$, $c_{mu}^k = 5mM$, $c_{ci}^k = 140mM$ we find $\frac{c_{ci}^{ca}}{c_{mu}^{ca}} \sim 10^{-9}$. Thus, with $c_{mu}^{ca} = 2mM$ and no Ca^{2+} influx into cilia, the exchangers are able to reduce the ciliary Ca^{2+} concentration much below nanomolar level. For $c_{ci}^{ca} \gg c_{mu}^{ca} \left(\frac{c_{ci}^{na}}{c_{mu}^{na}} \right)^4 \frac{c_{mu}^k}{c_{ci}^k}$ Eq. 15 can be approximated by the Michaelis-Menton form

$$j_{ci,mu}^{nckx} \approx \nu_{nckx} e^{r\phi_{ci}/2} \frac{c_{ci}^{ca}}{K_{nckx} + c_{ci}^{ca}}. \quad (18)$$

1.4 Signal transduction and cAMP dynamics

Since the focus of this paper is on the ion dynamics, we use a basic transduction model comprising odorant receptor, G protein, adenylate cyclase (AC), cAMP and CaMK [24, 25, 15]. After the activation of odorant receptors (or), a G protein (g) coupled transduction cascade leads to the activation of adenylate cyclase (ac) that synthesizes cAMP. We neglect diffusion of receptors, G-protein and AC in the membrane. Because experimental evidences suggests that the lifetime of an activated odorant receptor is less than a millisecond [26, 27], we use the steady state expression to model the fraction (with respect to the total concentration) of activated receptors or^* as a function of a slowly varying odorant stimulus $od(z, t)$, $or^* = \frac{od^{h_{od}}}{od^{h_{od}} + K_{od}^{h_{od}}}$. The equations for the fractions of

activated G protein and AC are

$$\begin{aligned}\partial_t g^* &= \beta_g \left((1 - g^*) \frac{or^*}{K_{or}} - g^* \right) \\ \partial_t ac^* &= \beta_{ac} \left((1 - ac^*) \frac{g^*}{K_g} - ac^* \right)\end{aligned}\tag{19}$$

where $0 \leq g^*(z, t) \leq 1$ and $0 \leq ac^*(z, t) \leq 1$. The rate constants β_g and β_{ac} determine the activation dynamics. Steady state fractions are $g^* = \frac{or^*}{or^* + K_{or}}$ and $ac^* = \frac{g^*}{g^* + K_g}$.

The synthesis of cAMP is stimulated by ac^* and is inhibited by Ca^{2+} via CaMK feedback. In first approximation, we model the CaMK feedback directly as a function of Ca^{2+} using an effective Hill equation. Thus, the cAMP concentration $c_{ci}^{camp}(z, t)$ satisfies the reaction-diffusion equation

$$\partial_t c_{ci}^{camp} = -\partial_z j_{ci}^{camp} + ac^* \frac{\alpha_{camp}^{max}}{1 + f_{camk}} - \beta_{camp} c_{ci}^{camp}\tag{20}$$

with $j_{ci}^{camp} = -\nu_{ci}^{camp} \partial_z c_{ci}^{camp}$ and $\nu_{ci}^{camp} = \frac{D_{camp}}{L_{ci}^2}$. The dimensionless parameter f_{camk} accounts for Ca^{2+} dependent inhibition of cAMP synthesis due to CaMK feedback,

$$\frac{d}{dt} f_{camk} = \beta_{camk} \left(\frac{f_{camk}^{max}}{1 + \left(\frac{K_{camk}}{c_{ci}^{ca}} \right)^{h_{camk}}} - f_{camk} \right).\tag{21}$$

1.5 Ciliary boundary conditions

The tip of a cilium at $z = 0$ is a sealed end with reflecting boundary conditions $j_{ci}^s(0, t) = 0$. At $z = 1$ the cilia are connected via the dendritic knob and the dendrite to the cell body. To reduce complexity, we refrain from implementing a spatial model for electrodiffusion in knob and dendrite. Instead, we use flux boundary conditions $j_{ci}^s(1, t) = j_{ci,cb}^s(t)$ where $j_{ci,cb}^s$ depends on concentration and voltage differences between cilia and cell body and on the ciliary and dendritic geometry. We do not use Dirichlet conditions $c_{ci}^s(1, t) = c_{cb}^s = \text{constant}$ because flux boundary conditions provide several advantages: First, flux conditions are flexible and allow to account for geometry diffusional constraints. Second, Dirichlet conditions can be retrieved from flux boundary conditions by changing parameters (see Fig. S4). Finally, with flux boundary conditions it is straightforward to map our spatial model into a well-stirred model that we use for parameter estimations (see next paragraph). In contrast, it is unclear how to consistently map a spatial model with constant Dirichlet boundary conditions into a well-stirred model with time dependent concentrations.

Because the diameters of knob and dendrite are not much different [28], we integrate the knob into the dendrite. We further assume that the dendrite acts like a radially well-stirred and homogeneous cylinder with no current leakage and voltage that changes linearly between cilia and the cell body. As a first approximation, we therefore use for $j_{ci,cb}^s$ an expression that is similar to the GHK formula for the flux through a channel [13],

$$j_{ci,cb}^s = \alpha_{ci,cb} \nu_{ci}^s e^{-\frac{(z_s \phi_{ci,cb})^2}{24}} \left(c_{ci}^s(1, t) e^{z_s \phi_{ci,cb}/2} - c_{cb}^s e^{-z_s \phi_{ci,cb}/2} \right),\tag{22}$$

with $\phi_{ci,cb}(t) = \phi_{ci}(1, t) - \phi_{cb}(t)$. The corresponding flux boundary condition for the ciliary potential ϕ_{ci} is

$$j_{ci,cb}^\phi = \frac{1}{C_{ci} U_T} \sum_s z_s I_{ci,cb}^s = \frac{V_{ci} \mathcal{F}}{C_{ci} U_T} \sum_s z_s j_{ci,cb}^s.\tag{23}$$

The dimensionless parameter $\alpha_{ci,cb}$ accounts for ciliary and dendritic properties. For example, for small $\alpha_{ci,cb}$ the flux between cilium and cell body is strongly restricted, whereas for large $\alpha_{ci,cb}$ we recover Dirichlet boundary conditions (see Fig. S4). To obtain a first estimation of $\alpha_{ci,cb}$, we assume that the overall dendritic flux J_{dend}^s can also be approximated as

$$J_{dend}^s = A_{dend} \frac{D_s}{L_{dend}} e^{-\frac{(z_s \phi_{ci,cb})^2}{24}} \left(c_{ci}^s(1, t) e^{z_s \phi_{ci,cb}/2} - c_{cb}^s e^{-z_s \phi_{ci,cb}/2} \right). \quad (24)$$

where L_{dend} and $A_{dend} = \pi R_{dend}^2$ are dendritic length and cross-section. With current conservation $J_{dend}^s = N_{ci} j_{ci,cb}^s V_{ci}$ and Eq. 22 we find

$$\alpha_{ci,cb} = \frac{1}{N_{ci}} \left(\frac{R_{dend}}{R_{ci}} \right)^2 \frac{L_{ci}}{L_{dend}}. \quad (25)$$

Eq. 25 has reasonable properties: ciliary efflux is facilitated for large A_{dend} and it is obstructed for large L_{dend} ; if many cilia converge into a dendrite, the efflux from a single cilium becomes restricted due to limitations of the flux through the dendrite. With $N_{ci} \sim 15$, $R_{dend}/R_{ci} \sim 10$ and $L_{ci}/L_{dend} \sim 1$ [28] we find $\alpha_{ci,cb} \sim 7$. We used this value for the simulations presented in the main text. However, a different value for $\alpha_{ci,cb}$ would not alter our conclusions from the main text, see Fig. S4.

2 Derivation of the well-stirred model

Starting from the spatial model we derived well-stirred equations that we used to fit experimental data and estimate parameters. The well-stirred equations are obtained by replacing $c_{ci}^s(z, t)$ with $c_{ci}^s(t)$ and $\partial_z j_{ci}^s(z, t)$ with $j_{ci,cb}^s(t)$. Thus, the well-stirred equations for concentrations and potential read

$$\begin{aligned} \frac{d}{dt} c_{ci}^s &= -j_{ci,cb}^s - j_{ci,mu}^s \\ \frac{d}{dt} c_{ci}^{ca} &= \frac{1}{1 + B_{ca}} \left(-j_{ci,cb}^{ca} - j_{ci,mu}^{ca} - \frac{d}{dt} c_{ci}^{cab} \right) \\ \frac{d}{dt} c_{ci}^{camp} &= ac^* \frac{\alpha_{camp}^{max}}{1 + f_{campk}} - \beta_{camp} c_{ci}^{camp} - j_{ci,cb}^{camp} \\ \frac{d}{dt} \phi_{ci} &= \frac{1}{C_{ci} U_T} \sum_s (-I_{ci,cb}^s - I_{ci,mu}^s). \end{aligned} \quad (26)$$

The rest of the equations remain formally unchanged.

3 Fitting procedure and derivation of a common set of parameters

In the biological scenario (referred to as the Cl^- scenario) amplification is due to Cl^- efflux, whereas in the artificial scenario (referred to as the Na^+ scenario) it is due to Na^+ influx. Because Na^+ and K^+ currents through CNG channels are small compared to the Ano2 current [17, 29, 30], we simplified and assumed that CNG channels are only permeable to Ca^{2+} . To minimize the differences between the two scenarios, in the Na^+ scenario we introduce artificial Ano2 channels that have the

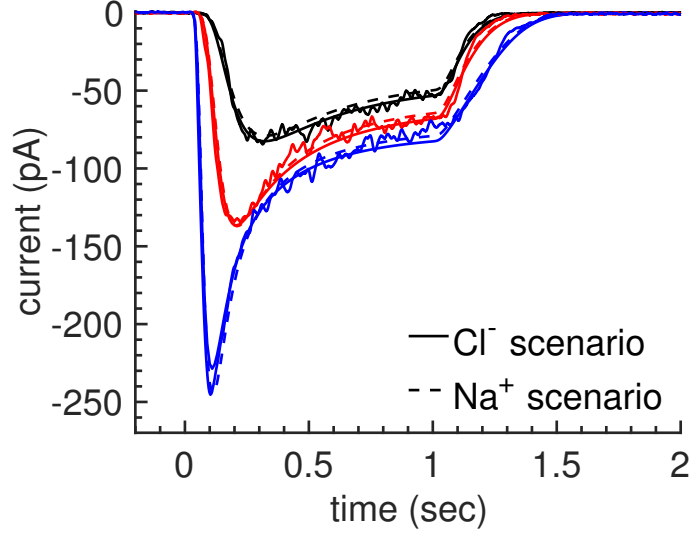


Fig. S1. Fitting with the well-stirred model. Odorant-induced responses (noisy traces) from a mouse olfactory receptor neuron to three different one second odorant stimulations with increasing concentrations (10, 30 and 100 μM) together with fitting results for each scenario.

same Ca^{2+} activation properties as the biological Ano2 channels but are permeable to Na^+ instead of Cl^- . In this way, both scenarios are absolutely identical except for the Ano2 permeability. To implement a Ca^{2+} -activated Na^+ conductance for Ano2 channels, we included the rate ν_{ano}^{na} , similarly to ν_{ano}^{cl} for the Cl^- conductance. Thus, in the Na^+ scenario we have ($\nu_{ano}^{cl} > 0$, $\nu_{ano}^{na} = 0$), whereas in the Cl^- scenario we have ($\nu_{ano}^{cl} = 0$, $\nu_{ano}^{na} > 0$). Because the driving forces for Cl^- and Na^+ are different, also the values for ν_{ano}^{na} and ν_{ano}^{cl} have to be different in order to obtain similar peak currents.

Whenever possible we used published values for our parameters (see Tables S1 and S2). For unknown parameters (e.g. most of the transduction parameters), we estimated values by calibrating the model to suction electrode recordings from a mouse ORN in response to a 1 s stimulation with three different odorant concentrations (Fig. S1, wiggled lines). Our goal was to find a common set of parameters that differs only in the values of ν_{ano}^{na} and ν_{ano}^{cl} but equally well fits the experimental data in both scenario. To compute such parameters, we generated two identical datasets by duplicating the experimental data. We then simultaneously fitted both datasets with the constraints $\nu_{ano}^{na} = 0$ for dataset 1, and $\nu_{ano}^{cl} = 0$ for dataset 2. In this way there was no procedural difference between the two scenarios. Because it is difficult to implement a fitting procedure based on the spatial model, we used the well-stirred model to perform the fitting using the Data to Dynamics (D2D) software [31, 32] based on maximum likelihood estimators. Fig. S1 shows that the data was equally well fitted in both scenarios. In a next step, we used the parameters (given in Tables S1 and S2) together with the assumption of homogeneous odorant stimulation along a cilium to perform simulations with our spatial model. We found that the parameters computed with the well-stirred model also provided a reasonably good fit to the data when used with the spatial model (see Fig. 2B in the main text).

4 Simulation results

We now present additional simulation results with strongest odorant stimulation (100 μM) that complement the results shown from the main text. Parameters are the same as in the main text, except for the ones that are modified as indicated in the figures.

In Fig. S2 we compare simulation results with dynamic K^+ concentration (black lines, see also Fig. 3 in the main text) to results with fixed K^+ concentration (dashed lines). The current is not much affected by holding K^+ constant (Fig. S2 a-b), but the dynamics of the Cl^- and Na^+ concentrations are strongly altered (Fig. S2c-f). For example, in the Cl^- scenario with fixed K^+ , Cl^- efflux does not entail a strong reduction in the ciliary Cl^- concentration due to electroneutrality requirements. Thus, affecting the K^+ dynamics also strongly affects the dynamics of Cl^- and Na^+ , showing that the ciliary ion dynamics are strongly coupled.

In Fig. S3 we compare simulation results between the two scenarios with the ciliary radius that is increased from 75nm to 750nm. Although the current is now strongly augmented due the 10-fold increased membrane surface, concentration changes are small due to the 100-fold larger volume. An effective model assuming constant ion concentrations would now be a valid approximation. Furthermore, with large radius the differences between the two scenarios are small and the advantages for amplification based on a Cl^- current disappear.

In Fig. S4 we compare simulation results obtained with $\alpha_{ci,cb} = 7$ (continuous lines) to results obtained with $\alpha_{ci,cb} = 100$ (dashed lines). $\alpha_{ci,cb} = 7$ is the default value that we used for simulations, and $\alpha_{ci,cb} = 100$ basically corresponds to Dirichlet boundary conditions (Fig. S4c-h). We find that the currents are only marginally affected by changing the boundary conditions (Fig. S4a-b). Dirichlet conditions slightly reduce the maximal fluctuations near the tip of a cilium, however, the main differences as discussed in the main text between Cl^- and Na^+ scenario persist (Fig. S4c-f). Thus, our conclusions from the main text are robust against reasonable changes in the boundary conditions.

Finally, in Fig. S5 we compare simulation results for the current obtained with the well-stirred model and mucosal concentrations of Na^+ and Cl^- that are reduced from 140 to 70 and 40 mM. Contrary to Fig. 5 in the main text, with the well-stirred model we do not observe bistability. This finding suggests that diffusion and concentration gradients play a crucial role for bistability. It is beyond the scope of this work to perform a detailed analysis of this phenomena. A comprehensive analysis would have to determine not only the parameter space where two fixed points exist, but also their stability and the position of the boundary (separatrix) that separates their basin of attraction. This task is highly complex due to the non-linear equations, diffusional properties, exchanger and channel characteristics, and biochemistry that affects the opening of CNG channels, and beyond the scope of this work.

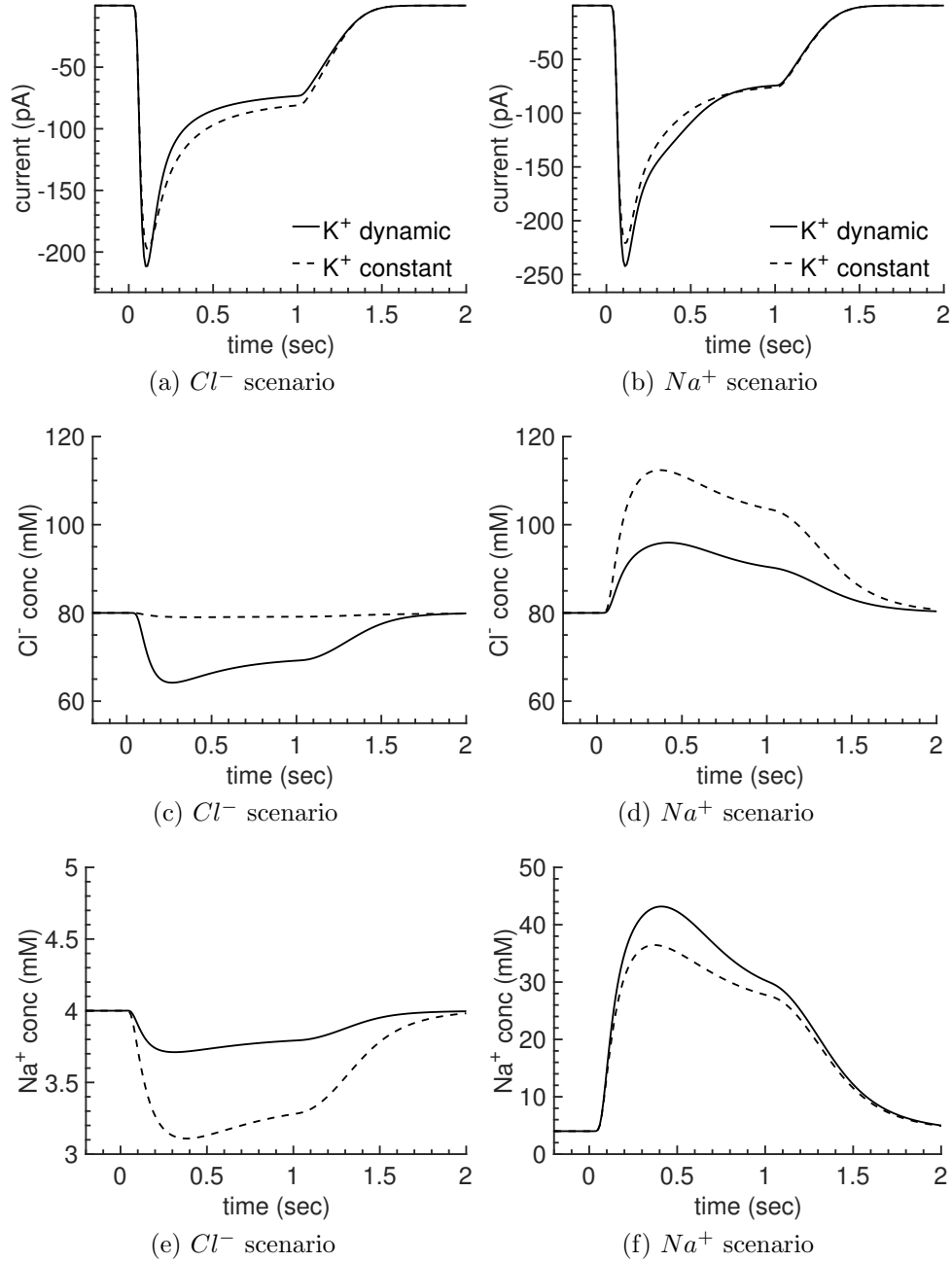


Fig. S2. Dynamic vs constant ciliary K^+ concentration. Comparison of simulation results with dynamic (solid lines) and fixed (dashed lines) K^+ concentration performed with the spatial model and strongest odorant stimulation ($100 \mu M$). The panels compare spatially averaged results for current, Cl^- and Na^+ concentrations (see also Fig. 3 in the main text).

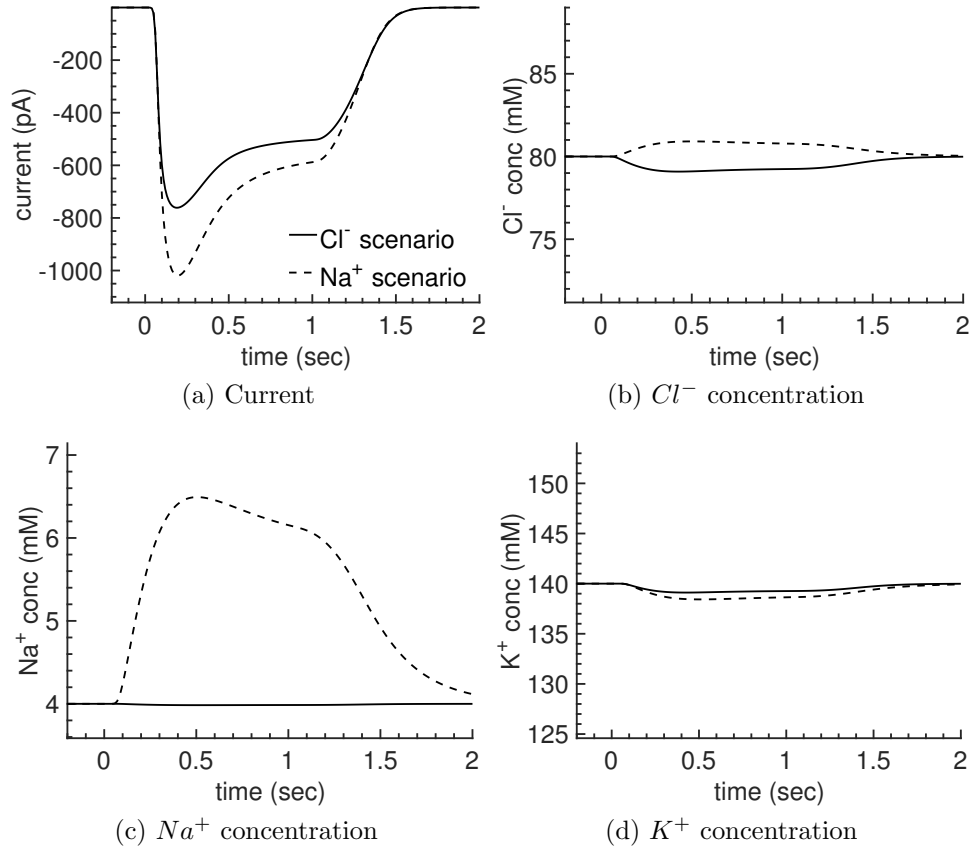


Fig. S3. Impact of a 10-fold increased ciliary radius. Simulations with 10-fold increased ciliary radius performed with the spatial model and strongest odorant stimulation. The panels compare spatially averaged results for current and ion concentrations between the two scenarios.

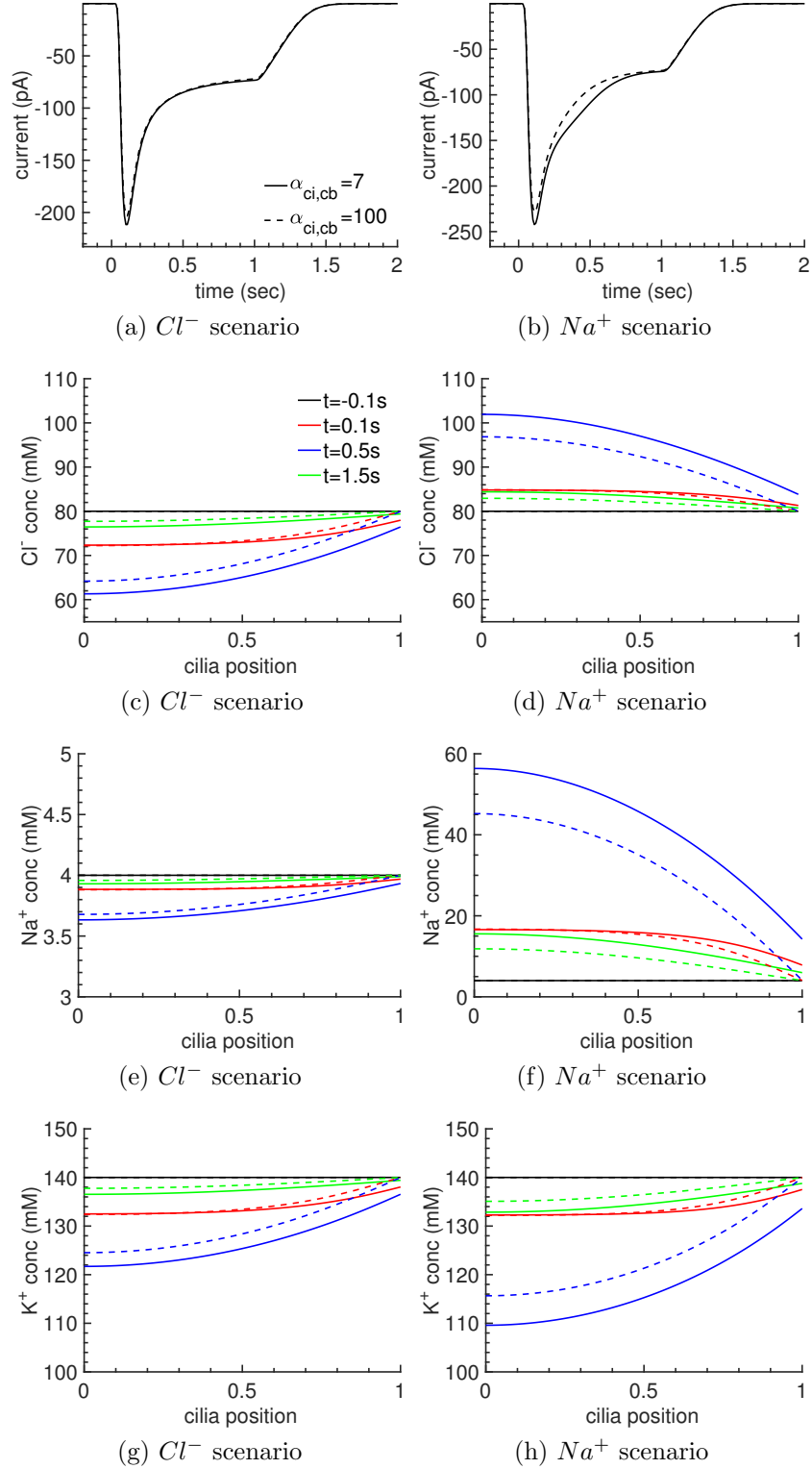


Fig. S4. Flux vs Dirichlet boundary conditions. Comparison of simulations results with $\alpha_{ci,cb} = 7$ (continuous lines) and $\alpha_{ci,cb} = 100$ (dashed lines) performed with the spatial model and strongest odorant stimulation. $\alpha_{ci,cb} = 7$ is the default value used for simulations. A value $\alpha_{ci,cb} = 100$ effectively corresponds to Dirichlet boundary conditions.

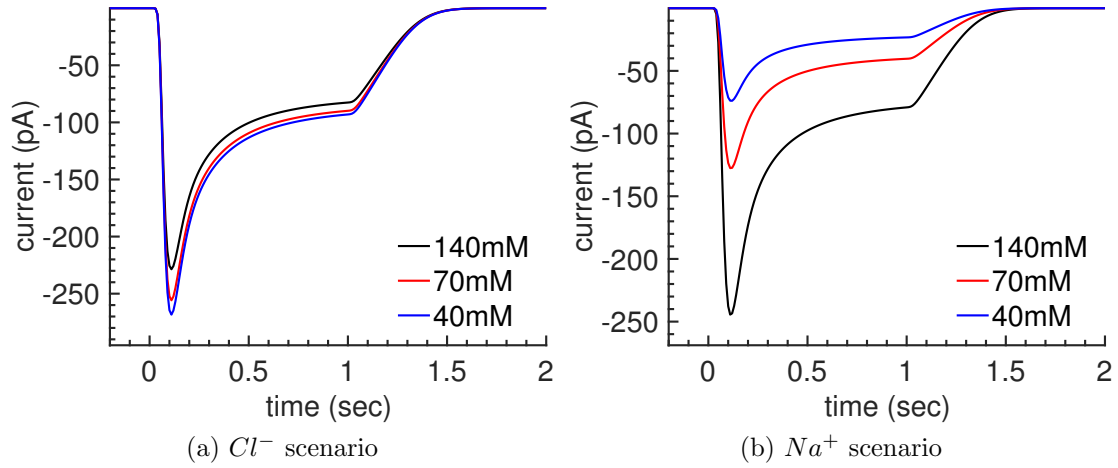


Fig. S5. Well-stirred simulations with reduced mucosal concentrations for Na^+ and Cl^- . Simulations are performed with the well-stirred model and strongest odorant stimulation ($100 \mu M$). Parameters are the same as in in Fig S1, except for the mucosal Na^+ and Cl^- concentrations that are indicated in the figure legend. Compared to the corresponding spatially resolved simulations in Fig. 5 in the main text, no bistability is observed with the well-stirred model.

5 Parameters used for the simulations

Description	Symbol	Unit	Value
Cilium length	L_{ci}	μm	25 [2]
Cilium radius	R_{ci}	μm	0.075 [2]
Cilia number	N_{ci}		15 [2]
Diffusion constants ($Ca^{2+}, Cl^{-}, Na^{+}, K^{+}$)	D_s	$\mu m^2/s$	220 [17], 2030, 1330, 1960 [33]
Mucus conc. (Ringer) ($Ca^{2+}, Cl^{-}, Na^{+}, K^{+}$)	c_{mu}^s	mM	2, 140, 140, 5
Cell body conc. ($Ca^{2+}, Cl^{-}, Na^{+}, K^{+}$)	c_{cb}^s	mM	40×10^{-6} , 80, 4, 140
Ano2 Ca^{2+} sensitivity	K_{ano}	μM	1.8 [34]
Ano2 Ca^{2+} Hill exponent	h_{ano}		2.3 [34]
Ano2 Cl^{-} permeability in the Cl^{-} scenario	ν_{ano}^{cl}	s^{-1}	7.6
Ano2 Na^{+} permeability in the Na^{+} scenario	ν_{ano}^{na}	s^{-1}	3.4
CNG cAMP sensitivity	K_{cng}^{min}	μM	4 [35, 36]
CNG cAMP Hill exponent	h_{cng}		1.8 [35]
CNG Ca^{2+} permeability	ν_{cng}^{ca}	s^{-1}	0.5
CNG Na^{+} permeability	ν_{cng}^{na}	s^{-1}	0
CNG K^{+} permeability	ν_{cng}^k	s^{-1}	0
CNG Ca^{2+} feedback sensitivity	K_{cng}^{ca}	μM	10 [20]
CNG Ca^{2+} feedback max	f_{cng}^{ca}		4 [20]
NCKX4 rate constant	ν_{nckx}	s^{-1}	1.2
NCKX4 Michaelis constant	K_{nckx}	μM	22
Rate constant for efflux from a cilium	$\alpha_{ci,cb}$	s^{-1}	7
Cell body leak voltage	U_{cb}^{leak}	mV	-65
Cell body leak conductance	g_{cb}^{leak}	nS	20
Cell body capacitance	C_{cb}	nF	10^{-3}
Ciliary membrane capacity	C_m	$nF/\mu m^2$	10^{-5}

Table S1. Parameters for electrodiffusion.

Description	Symbol	Unit	Value
cAMP diffusion constant	D_{camp}	$\mu m^2/s$	270 [37, 38]
cAMP maximal synthesis rate	α_{camp}^{max}	$\mu M s^{-1}$	95
cAMP hydrolysis rate	β_{camp}	s^{-1}	50
cAMP cell body concentration	c_{cb}^{camp}	μM	0
CaMK time constant	β_{camk}	s^{-1}	0.7
CaMK max inhibition	f_{camk}^{max}		28
CaMK Ca^{2+} sensitivity	K_{camk}	μM	2
CaMK Ca^{2+} Hill exp	h_{camk}		3
Receptor sensitivity	K_{od}	μM	45
Receptor Hill exponent	h_{od}		2
G protein sensitivity	K_{or}		0.7
G protein time scale	β_g	s^{-1}	6.4
AC time scale	β_{ac}	s^{-1}	20
AC sensitivity	K_g		0.1

Table S2. Parameters for the biochemical transduction model.

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