Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} current ensures robust and reliable signal amplification in vertebrate olfactory receptor neurons

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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\textsuperscript{−}. 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\textsuperscript{+}, Ca\textsuperscript{2+}, K\textsuperscript{+}, and Cl\textsuperscript{−} 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\textsuperscript{−} or Na\textsuperscript{+} currents. We found that amplification produced by Na\textsuperscript{+} influx instead of a Cl\textsuperscript{−} efflux is problematic for several reasons: First, the Na\textsuperscript{+} current amplitude varies greatly, depending on mucosal ion concentration changes. Second, a Na\textsuperscript{+} current leads to a large increase in the ciliary Na\textsuperscript{+} concentration during an odorant response. This increase inhibits and even reverses Ca\textsuperscript{2+} clearance by Na\textsuperscript{+}/Ca\textsuperscript{2+}/K\textsuperscript{+} exchange, which is essential for response termination. Finally, a Na\textsuperscript{+} current increases the ciliary osmotic pressure, which could cause swelling to damage the cilia. By contrast, a transduction pathway based on Cl\textsuperscript{−} efflux circumvents these problems and renders the odorant response robust and reliable.

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. 1A). 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. 1B).

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. 1B), 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 Ca\textsuperscript{2+} and to a lesser extent to Na\textsuperscript{+} and K\textsuperscript{+} (3, 4). The Ca\textsuperscript{2+} influx triggers the opening of a secondary anionic channel: the Ca\textsuperscript{2+}-gated Cl\textsuperscript{−} channel Anoctamin 2 (Ano2), also known as TMEM16B (5–8). Cl\textsuperscript{−} efflux ensues, as intracellular Cl\textsuperscript{−} is high such that the Nernst equilibrium potential for Cl\textsuperscript{−} is positive to the ORN resting potential (9, 10). Ciliary Ca\textsuperscript{2+} is predominantly removed by electrogenic Na\textsuperscript{+}/K\textsuperscript{+}/Ca\textsuperscript{2+} exchange (NCKX4) (11). The activation of the Ano2 channels is the main amplification step, and most of the transduction current is carried by Cl\textsuperscript{−} efflux (12–16). This large amplification is thought to be the principal function of the Cl\textsuperscript{−} current. When the Cl\textsuperscript{−} 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\textsuperscript{−} 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\textsuperscript{+} and Ca\textsuperscript{2+}, which would disturb Ca\textsuperscript{2+} homeostasis and affect several Ca\textsuperscript{2+}-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\textsuperscript{+} and Ca\textsuperscript{2+} concentrations with currently unclear consequences. For example, robustness of Ca\textsuperscript{2+} clearance could be affected because the intracellular Ca\textsuperscript{2+} concentration would increase due to larger osmotic pressure, which could cause swelling to damage the cilia. By contrast, a transduction pathway based on Cl\textsuperscript{−} efflux circumvents these problems and renders the odorant response robust and reliable.

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Ca\(^{2+}\) influx, and Ca\(^{2+}\) extrusion via Na\(^{+}/K^{+}/Ca^{2+}\) exchange would be inhibited due to a higher intracellular Na\(^{+}\) concentration. These problems could conceivably be obviated by activating a current component based on Cl\(^{-}\) influx. In addition, a current based on Cl\(^{-}\) influx might as well reduce response variation caused by changes in mucosal ion concentrations, contrary to a current based on Na\(^{+}\) influx (24, 25). Indeed, the cilia of ORNs must function reliably while embedded in mucus exposed to the outside world. Mucosal ion concentrations can be altered by exposure to water or sneezing, as well as by stimulation of the parasympathetic and \(\beta\)-adrenergic systems in mice or human patients with airway disease (e.g., cystic fibrosis) (26).

Since no experimental method presently allows for the measurement of ion concentrations in the small volume of a cilium, we used mathematical modeling and response simulation to address the role of the Cl\(^{-}\) current. We developed a model of olfactory transduction that not only incorporates the biochemically transduction pathway but also includes spatial resolution of ion dynamics in the volume of the cilium during an odorant response. To study the impact of the current carrier, we modeled and compared signal amplification for two complementary scenarios: a biological scenario where amplification is based on a Cl\(^{-}\) current and an artificial scenario where amplification is based on a Na\(^{+}\) current. One possibility to model the artificial scenario would have been to remove the Ano2 channels and increase the Na\(^{+}\) current by simply augmenting the number of CNG channels. However, this would have required additional substantial changes in the transduction part of the model to adjust the cAMP dynamics such that odorant responses with a CNG channel-based current would be comparable to those produced by a Cl\(^{-}\) channel-based current. We therefore have elected to keep the CNG channels unchanged and to postulate a Ca\(^{2+}\)-activated Na\(^{+}\) current, similar to the Ca\(^{2+}\)-activated Cl\(^{-}\) current. We therefore have elected to keep the CNG channels unchanged and to postulate a Ca\(^{2+}\)-activated Na\(^{+}\) current, similar to the Ca\(^{2+}\)-activated Cl\(^{-}\) current. In this way, we could keep all aspects of the transduction pathway unchanged between the two scenarios, and we could neatly compare the effects of amplification based on Na\(^{+}\) influx and Cl\(^{-}\) efflux without complicating factors. Our general conclusions apply, however, equally well to any increase in Na\(^{+}\) influx regardless of the mechanism of activation of the Na\(^{+}\) current. We show that signal amplification based on Na\(^{+}\) influx can produce responses comparable to those produced by Cl\(^{-}\) efflux, but a mechanism based on Na\(^{+}\) renders the response much less stable to changes in mucosal ion concentrations. Moreover, a Na\(^{+}\) current leads to a large increase in intracellular Na\(^{+}\), which inhibits and can even reverse Ca\(^{2+}\) extrusion by Na\(^{+}/Ca^{2+}/K^{+}\) exchange. Finally, a Na\(^{+}\) current leads to an increase in the ciliary osmotic pressure, which could conceivably produce swelling that damages the cilia. All of these detrimental consequences are avoided by the use of a Cl\(^{-}\) current, which not only amplifies the response but also keeps it robust and reliable.

**Results**

We compare two complementary scenarios: In the biological scenario (in the following referred to as the Cl\(^{-}\) scenario) amplification is due to Cl\(^{-}\) efflux, whereas in the artificial scenario (referred to as the Na\(^{+}\) scenario) amplification is due to Na\(^{+}\) influx (Fig. 2). To minimize the differences between the two scenarios, in the Na\(^{+}\) scenario we introduce artificial Ano2 channels that have the same Ca\(^{2+}\) activation properties as the biological Ano2 channels but are permeable to Na\(^{+}\) instead of Cl\(^{-}\). For simplicity, we also refer to them as Ano2 channels, since from the scenario it becomes clear which type is considered. Hence, both scenarios are absolutely identical except for the Ano2 permeability. We further simplify and neglect the Na\(^{+}\) and K\(^{+}\) currents through CNG channels because they are small compared with the Ano2 current (13, 14, 21) and therefore only have a minor impact on ion dynamics. Moreover, in this way both scenarios are clearly distinguished, and differences can be unambiguously attributed to effects evoked by the two current carriers.

**A Na\(^{+}\) Current Induces Large Na\(^{+}\) Fluctuations in a Cilium.** We calibrated our model by fitting suction-electrode recordings from a mouse ORN in response to a 1-s stimulation with three different odorant concentrations (for details see SI Appendix). In Fig. 2B we compare the computed current between mucus and 15 identical cilia with the experimental data. The simulations
scenario. The distributions correspond to Figs. 2 and 3 for the \( \nu^- \) and \( \nu^+ \) electrolyte permeabilities. In the \( \nu^- \) scenario, but \( \nu^+ = 0 \), the Na\(^+\) influx along a cilium at four different times. The spatial distributions along a cilium at four different times. The \( \nu^- \) scenario we have (Fig. 3A, solid lines and dashed lines those fluxes 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 cilary 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 cilary 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. 4A and B) and to a lesser extent for the K\(^+\) concentration (Fig. 4C). With Cl\(^-\) as the current carrier, almost

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**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.

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**Fig. 4.** Spatial distributions along a cilium at four different times. The rescaled cilary 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 \( \mu \text{M} \)).
no Na\(^+\) gradient is generated during an odorant response. This is in strong contrast to a Na\(^+\) current that generates large Na\(^+\) gradients. At the tip of a cilium, Na\(^+\) influx pushes the Na\(^+\) concentration to values that are almost 15-fold larger compared with the basal value of 4 mM (Fig. 4A, blue dashed line). As a consequence, the NCKX4 exchangers are inhibited, leading to reduced Ca\(^{2+}\) efflux along the cilium in the Na\(^+\) scenario (Fig. 4E). The Cl\(^-\) gradients have opposite behaviors in the two scenarios (Fig. 4B). In the Cl\(^-\) scenario, Cl\(^-\) efflux leads to a distribution where the concentration is minimal near the cilium tip. In contrast, a Na\(^+\) current leads to a Cl\(^-\) increase that is maximal near the tip as Cl\(^-\) flows into the cilia from the cell body due to the cilial depolarization (Fig. 4F). The K\(^+\) concentration decreases toward the tip of a cilium, but the K\(^+\) gradients are qualitatively similar between the two scenarios; however, a Na\(^+\) current entails much higher K\(^+\) gradients (Fig. 4C). In contrast to the strong gradients generated for Cl\(^-\), Na\(^+\), and K\(^+\), the Ca\(^{2+}\) concentration remains rather homogeneous along a cilium during the odorant response (Fig. 4D). This is because we assumed that a cilium is uniformly activated by the odorant application, and NCKX4 exchangers that remove Ca\(^{2+}\) are uniformly distributed along a cilium. Because we did not include spontaneous receptor activity and a basal cAMP synthesis rate, the resting cAMP concentration vanishes and there is no Ca\(^{2+}\) influx via CNG channels in the absence of odorant stimulation. At rest the exchangers therefore reduce the ciliary Ca\(^{2+}\) concentration much below nanomolar level (SI Appendix). The Ca\(^{2+}\) gradient at \(t = -0.1\) s in Fig. 4D (black trace) is caused by influx from the cell body where the concentration is 40 mM. Note that at \(t = 1.5\) s (green trace), the Ca\(^{2+}\) concentration did not yet regain its resting distribution. The subtle discrepancies in the Ca\(^{2+}\) dynamics between the two scenarios are generated by inhibition of NCKX4 exchange in the Na\(^+\) scenario (Fig. 4E). Ultimately, this is the reason for the differences in the Ano2 currents between the two scenarios in Fig. 2A. One might be surprised that the generated Ca\(^{2+}\) discrepancies are not larger. However, the parameters are specifically fitted such that the currents in both scenarios agree with the experimental data. Since the currents reflect the Ano2 open probability that depends very sensitively on Ca\(^{2+}\) with a Hill exponent of 2.3 (8), the Ca\(^{2+}\) concentrations necessarily have to be very similar between the two scenarios. As we show below, this is no longer the case when we modify parameters, for example by changing mucosal ion concentrations.

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. 5A 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. 5A). 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. 5B). 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 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. 5B, blue line). Correspondingly, intracellular ion concentrations attain plateau values that are very different from their initial values (Fig. 5C). 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. 5D, 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. 5B, 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...
exchange is electrogenic and depends on the Na\(^{+}\) and K\(^{+}\) gradients between cilia and mucus. We compared simulations for two complementary scenarios: a biological scenario where signal amplification is based on Cl\(^{-}\) efflux and an artificial scenario where it is based on Na\(^{+}\) influx. We found significant differences between both scenarios, which offer explanations for why a Cl\(^{-}\) current is preferential for signal amplification in ORNs.

Most important for reliable signaling in a varying nasal environment, our simulations reveal that a current carried by Cl\(^{-}\) efflux is largely insensitive to Cl\(^{-}\) and Na\(^{+}\) concentration changes in the mucus, contrary to a current carried by Na\(^{+}\) influx. This result confirms previous speculations that a Cl\(^{-}\) current in ORNs might have evolved to render the odorant response robust against mucosal ion concentration changes (24, 25).

Our work revealed complex and highly coupled ion dynamics in a cilium. We found striking differences between the two scenarios, in particular for the dynamics of the Cl\(^{-}\) and Na\(^{+}\) concentrations. With Cl\(^{-}\) efflux, the ciliary Cl\(^{-}\) concentration decreases, as expected, but surprisingly the Na\(^{+}\) concentration remains largely unaffected. In contrast, with Na\(^{+}\) influx, both Cl\(^{-}\) and Na\(^{+}\) concentrations significantly increase during an odorant response. These differences have two major consequences: First, the large increase of the Na\(^{+}\) concentration in the Na\(^{+}\) scenario reduces the Na\(^{+}\) gradient between mucus and cilium that drives NCKX4 exchange. NCKX4 inhibition reduces the rate of Ca\(^{2+}\) clearance, which affects response termination via the closure of the Ca\(^{2+}\)-activated Ano2 channels and other transduction processes that depend on Ca\(^{2+}\) feedback (1). NCKX4 inhibition does not occur in the Cl\(^{-}\) scenario where the Na\(^{+}\) concentration remains low such that fast Ca\(^{2+}\) clearance is ensured. Second, whereas the osmotic concentration in a cilium decreases with a Na\(^{+}\) current, it increases with a Na\(^{+}\) current. This might be of relevance because an increase in osmotic concentration necessarily entails water influx that might compromise the stability of the fragile cilium.

In line with steady-state results obtained previously by Lindemann (34), we found that large ciliary concentration gradients for Cl\(^{-}\) and K\(^{+}\) are generated in both scenarios. Unexpectedly, with a Cl\(^{-}\) current almost no Na\(^{+}\) gradient is generated, which ensures homogeneous Ca\(^{2+}\) clearance along the whole cilium. In contrast, a large Na\(^{+}\) gradient is generated in the Na\(^{+}\) scenario, which inhibits and can even reverse exchanger function in the frontal part of a cilium.

These differences between both scenarios are generic and a consequence of the current carrier (but see next paragraph). They occur independently of the precise type of Cl\(^{-}\) or Na\(^{+}\) channels that are involved. Thus, signal amplification based on increasing the number of CNG channels would suffer from the same problems generated by a large Na\(^{+}\) influx. Moreover, since CNG channels are permeable to Ca\(^{2+}\) and Na\(^{+}\), increasing their number would increase Ca\(^{2+}\) influx and at the same time reduce Ca\(^{2+}\) clearance by NCKX4 exchange due to increased intracellular Na\(^{+}\). This has the potential to strongly alter the Ca\(^{2+}\) dynamics. Robust Ca\(^{2+}\) homeostasis is, however, essential because of multiple Ca\(^{2+}\)-dependent feedback processes in the biochemical transduction pathway.

We found a striking effect that is specific to the fact that we introduced an artificial Na\(^{+}\) channel that is Ca\(^{2+}\)-activated. With such a channel type we found a bistable system when we reduced the mucosal Na\(^{+}\) concentration. In this case, the reduced Na\(^{+}\) driving force together with ciliary depolarization and decreasing K\(^{+}\) and increasing Na\(^{+}\) concentrations inhibited and reversed the exchanger mode in the frontal part of a cilium at a strong stimulation. Part of the exchangers now import Ca\(^{2+}\) from the mucus, which keeps the Ca\(^{2+}\)-activated Na\(^{+}\) channels open and the current flowing even after the odorant stimulation is over. Thus, an olfactory pathway based on Ca\(^{2+}\)-activated Na\(^{+}\) channels would pose a serious threat to robustness and reliability of odorant detection. Interestingly, although Ca\(^{2+}\)-activated K\(^{+}\) channels are ubiquitous and important to control neuronal excitability and many other physiological processes (35, 36), Ca\(^{2+}\)-activated Na\(^{+}\) channels seem much less prevalent. We found only very few reports where such channels produce long-lasting action potentials in starfish oocyte or the egg of a nemertean worm (37).

Since Ca\(^{2+}\) clearance is often accomplished by Na\(^{+}\)-dependent exchangers, the rare occurrence of such channels might be related to the bistable behavior observed here.

Because of the large number of parameters and nonlinear interactions, it is beyond the scope of this work to present a comprehensive analysis and discussion of the parameter space. Our goal here was to outline the main differences between the Cl\(^{-}\) and Na\(^{+}\) scenarios, and these differences are robust against reasonable changes in parameter values.

We developed a detailed spatiotemporal model that we applied to analyze ion dynamics in a cilium. However, our model can also be applied to study other puzzling aspects of the odorant response, e.g., the role of PDE 1C in response termination (38, 39). It can be applied to clarify the effect of cAMP clearance via diffusion into the cell body vs. PDE hydrolysis by PDE 1C. In addition, our model can be adapted to study other biological systems with constrained spaces, e.g., outer dendrite and sensilla in insect olfaction, microvilli of insect photoreceptors, taste cells, or synapses and synaptic buttons (2). Each of these systems has to balance its functional need for ionic currents with the advantages or limitations of having to operate with a small intracellular volume. In olfactory cilia, this is achieved by combining a cationic current with a secondary anionic current to ensure response amplification that preserves response stability and reliability.

**Materials and Methods**

**Single-Cell Recordings of Odorant-Induced Responses.** Mice were handled and killed in accordance with methods approved by the Monell Chemical Senses Center Institutional Use and Care Committee. Mice were killed with CO\(_2\) followed by cervical dislocation. The suction-pipette technique (40, 41) was used to record odorant-induced responses from isolated ORNs. In short, the cell body of an isolated ORN is sucked into the tip of a recording pipette. The cilia remain outside of the pipette and therefore accessible for solution changes and odorant stimulation. No access is gained to the intracellular environment, and thus intracellular ion concentrations remain unperturbed. Also, the intracellular voltage is free to vary, as the ORN is not voltage clamped. The current recorded in this configuration is the current carried by the ORN transduction channels and exits via the cell body. Currents were recorded with a Warner PC-501A patch clamp amplifier (Warner Instruments), filtered dc to 50 Hz, and digitized at 10 kHz using a Power1401 MK2 acquisition board and Signal software (Cambridge Electronic Design). Fast solution exchanges were achieved by moving the ORN in the tip of the recording pipette across the interface to two parallel streams of solution, using the Perfusion Fast-Step system (Warner Instruments). Ringer solution contained 140 mM NaCl, 5 mM KCl, 1 mM MgCl\(_2\), 2 mM CaCl\(_2\), 0.01 mM EDTA, 10 mM Hepes, and 10 mM glucose. The pH was adjusted to 7.5 with NaOH. ORNs were stimulated with an odorant mix of cineole and acetylphene.

**Computational Model.** We constructed a spatially resolved mathematical model to analyze odorant responses in vertebrate ORNs. Our model accounts for the biochemical transduction pathway (Fig. 1B) and the spatiotemporal ion dynamics in the cilium during an odorant response. A schematic of the electrical part of our ciliary model is shown in Fig. 2A, and for details we refer the interested reader to SI Appendix.

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