Perspectives on: Information and coding in mammalian sensory physiology

Response kinetics of olfactory receptor neurons and the implications in olfactory coding

Johannes Reisert[1] and Haiqing Zhao[2]

[1]Monell Chemical Senses Center, Philadelphia, PA 19104
[2]Department of Biology, Johns Hopkins University, Baltimore, MD 21218

Olfaction begins with the detection of odorants by olfactory receptor neurons (ORNs) in the nasal cavity. Olfactory transduction is mediated by a G protein–coupled transduction cascade culminating in the opening of the two olfactory transduction ion channels, the olfactory CNG channel and the Ca^{2+}-activated Cl^- channel anoctamin 2 (Ano2), and ultimately action potential (AP) generation. The mechanisms that activate olfactory transduction have been understood quite well over the last two decades. Mechanisms of response adaptation, however, have actually become much less clear, with mechanisms previously thought to be important now suggested to play less significant roles, raising the question of which transduction components are the target of adaptational feedback. Because ORNs are often stimulated rhythmically by the inhalation of odorants, fast response termination should be a prerequisite to adequately resolve the temporal aspect of the stimulus. Recent progress suggests that mechanisms that regulate ciliary Ca^{2+} transients dictate kinetics of transduction termination. Ultimately, the question to answer is how ORNs code for "natural" stimuli in the behaving animal.

Activation and amplification of olfactory transduction

ORNs are embedded in the olfactory epithelium that covers the olfactory turbinates, a convoluted bone structure, located in the posterior nasal cavity in most mammals. These primary sensory neurons, like touch-sensitive neurons but unlike photoreceptors (see accompanying Perspectives by Bautista and Lumpkin, 2011, and Schwartz and Rieke, 2011) generate APs upon stimulation and send axons to the olfactory bulb, where they communicate with mitral and tufted (second-order) neurons. Odorants are carried to ORNs via the inhaled air in a rhythmic fashion, the frequency of which depends on the breathing frequency that can vary from 2 to 3 Hz at rest to up to 10 Hz during sniffing in rodents (Youngentob et al., 1987; Tankersley et al., 1994; Kepecs et al., 2007).

Olfactory transduction occurs in olfactory cilia, which extend from the dendritic knob at the end of the single dendrite into the mucus that covers the epithelium (Fig. 1 A). Transduction begins with the binding of odorants to odorant receptors (ORs) located in the ciliary membrane (Fig. 1 B). A given ORN selectively expresses one of ~1,000 types of functional ORs found in the mouse genome. The OR triggers the activation of the olfactory G protein G_{olf}, which in turn activates adenylyl cyclase III (ACIII), leading to an increase in ciliary cAMP. Subsequent opening of the olfactory CNG channel by cAMP leads to an influx of Na^+ and Ca^{2+}, with Ca^{2+} gating a Ca^{2+}-activated Cl^- channel (Kleene, 2008; Reisert and Restrepo, 2009; Su et al., 2009). As intracellular Cl^- is high in ORNs, a secondary excitatory anionic current ensues and carries up to 90% of the odorant-induced receptor current (Lowe and Gold, 1993; Reisert et al., 2005; Boccaccio and Menini, 2007). Fig. 2 shows the currents generated in an ORN that has been loaded with caged 8-Br-cAMP. Upon photolysis (Fig. 2, arrow) uncaged 8-Br-cAMP leads to activation of the CNG channel, giving rise to an initial small current that is followed by a large secondary current. This secondary current can be suppressed by niflumic acid, a blocker of the Ca^{2+}-activated Cl^- channel.

The importance of the G_{olf} α subunit, ACIII, and the principle subunit of the CNG channel, CNGA2, in response to activation was demonstrated in mice engineered to lack these transduction components. Such mice showed hardly any odorant responses, and pups had poor survival rates because of their inability to feed. Although the knockout approach was very successful to demonstrate that these transduction components are required for olfactory transduction, the lack of response precluded more detailed analysis of their role in response kinetics. The physiological role of the last

Correspondence to Johannes Reisert: jreisert@monell.org

Abbreviations used in this paper: ACIII, adenylyl cyclase III; Ano2, anoctamin 2; AP, action potential; CaM, calmodulin; CaMKII, CaM-dependent kinase II; NCKX4, Na^+/Ca^{2+} exchanger 4; OMP, olfactory marker protein; OR, odorant receptor; ORN, olfactory receptor neuron; PDE, phosphodiesterase.

The Rockefeller University Press
J. Gen. Physiol. Vol. 138 No. 3 303–310
www.jgp.org/cgi/doi/10.1085/jgp.201110645

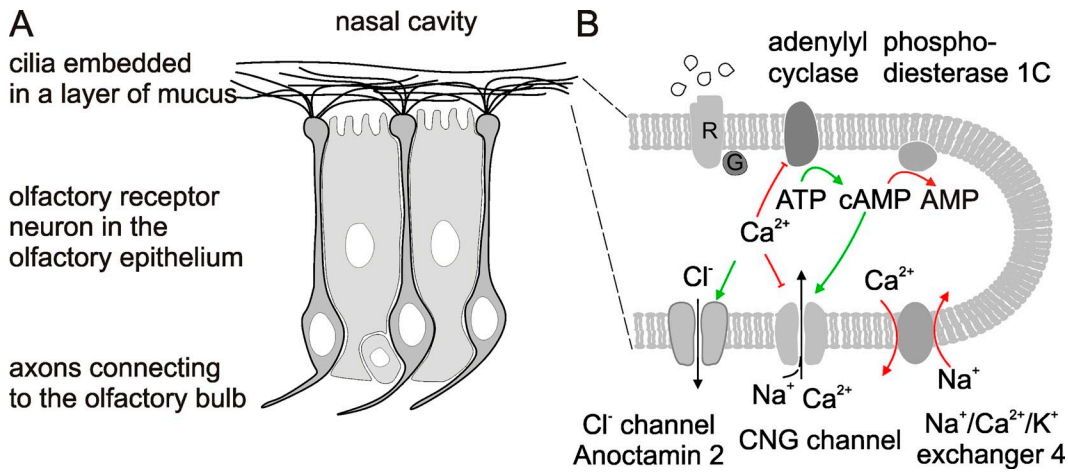

Figure 1. Olfactory transduction. (A) ORNs are embedded in the olfactory epithelium together with sustentacular and basal cells. They are bipolar neurons, sending an axon to the olfactory bulb and extending a single dendrite to the epithelial border. The dendrite terminates in the dendritic knob, from which cilia radiate into the mucus that lines the nasal cavity. (B) Cross section through the tip of an olfactory cilium that contains the olfactory G protein–coupled transduction cascade. "R" and "G" denote the seven-transmembrane OR and the olfactory G protein, G_{olf}. Green and red arrows indicate transduction mechanisms involved in activating or terminating/adapting the odorant-induced response.

component in the activation chain, the olfactory Ca^{2+}-activated Cl^- channel, has been known since the early 1990s but was only molecularly identified in 2009 to be Ano2 (Stephan et al., 2009; Hengl et al., 2010; Rasche et al., 2010). A recent loss-of-function study confirmed that Ano2 is indeed the olfactory Ca^{2+}-activated Cl^- channel. Intriguingly, Ano2 knockout mice showed no behavioral deficit performing some Go/NoGo tasks (Billig et al., 2011), although humans with Ano2 mutations may suffer olfactory defects (Stephan et al., 2009). Although, again, knocking out the Cl^- channel will preclude a detailed investigation of its contribution to receptor current regulation, it will possibly reveal in more detail what the kinetics of the cAMP-induced current might be, as this will be the only remaining current.

Odorant-induced APs

An increase in odorant concentration triggers, in a graded manner, APs with a shorter delay after stimulus onset and a higher firing rate. Response delays can be as short as ~30 ms and maximal AP firing rates reach up to around 200 Hz in isolated mouse ORNs at saturating odorant concentrations. The dynamic range of ORNs is typically narrow, saturating within 2 log units of odorant concentration above threshold (Reisert and Matthews, 2001; Rospars et al., 2008). The number of APs fired does not show a monotonic behavior. Instead, the number of APs only increases up to intermediate odorant concentrations and decreases thereafter at higher concentrations down to only two to three APs. These APs are generated at the very onset of odorant stimulation during the rising phase of the receptor current (Shibuya and Shibuya, 1963; Gesteland and Sigwart, 1977; Reisert and Matthews, 2001; Reisert et al., 2007; Rospars et al., 2008). The odorant-induced receptor current can persist for much longer at higher odorant concentration for as long as the odorant is present with no further AP generation (see Fig. 3). Thus, under these conditions, no signaling of the presence of odorants would be sent to the brain by this particular ORN. Even a further increase in odorant concentration applied while a receptor current is still present will increase the receptor current but not cause additional AP firing (Reisert and Matthews, 1999). The shortening of the spike train is caused by a

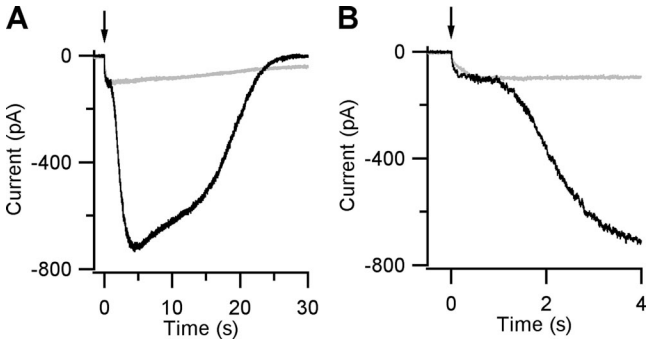

Figure 2. Sequential activation of the olfactory CNG and Cl^- transduction channels. (A) Receptor currents from a mouse ORN recorded using the whole cell patch-clamp technique. The ORN was loaded with the photolysable compound caged 8-Br-cAMP via the recording pipette. Exposure to a short UV flash (arrow) caused the release of the "cage" from 8-Br-cAMP, which triggered the opening of the CNG channel and a small initial Na^+ and Ca^{2+} current. After a clear delay (in this particular ORN), an additional current was generated that could be blocked by the Ca^{2+}-activated Cl^- channel blocker, niflumic acid. (B) Same data as in A but on an expanded time scale to show the pronounced sequential activation of the two currents. Modified from Boccaccio and Menini (2007) with permission from the authors and the American Physiological Society.

progressive decline of the AP amplitude (Fig. 3 A, inset), probably by inactivation during prolonged depolarization of voltage-gated Na^+ and Ca^{2+} channels, which generate the olfactory AP (Trotier, 1994; Kawai et al., 1997). Thus, AP generation in ORNs and the duration of the spike train seem to be primarily controlled by the speed of activation (slope) of the receptor current rather than its overall time course. But termination of the receptor current is important for odorant perception during repeated stimulation, as shown in Fig. 3. ORNs were exposed twice in rapid succession for 1 s to the phosphodiesterase (PDE) inhibitor IBMX, which leads to an increase in ciliary cAMP and thus receptor current generation. In this kind of double-pulse stimulation paradigm, ORNs can only reliably generate APs to the second stimulation if the receptor current to the first odor stimulus has entirely terminated to allow the ORN to fully hyperpolarize to its resting membrane potential. For short interstimulus times (e.g., 0.25 s; Fig. 3, A and E, for time dependence of AP firing recovery), only around 50% of wild-type ORNs generate APs in response to the second stimulation but reliably do so when the interpulse time is lengthened to 1 s (Fig. 3 E). Or in other words, an increase in stimulation frequency (shorter interpulse time) progressively abolished AP generation.

Even a small response prolongation caused by altering the transduction cascade by removing a CaM-binding site on the CNG channel ($CNGB1^{\Delta CaM}$ mice; see below) can significantly alter AP firing during repetitive stimulation. Such $CNGB1^{\Delta CaM}$ ORNs were even less likely to generate APs compared with wild-type mice to the second stimulation at short (0.25-s) interpulse times (Fig. 3, B and E) because of the subtle response prolongation of the first response; but they can do so again once the interpulse interval is lengthened (Fig. 3 D). Furthermore, these changes are also conveyed to the olfactory bulb. Local field potential recordings from olfactory bulbs of freely breathing mice showed equivalent reductions in response magnitude to a second odorant exposure in $CNGB1^{\Delta CaM}$ mice compared with wild-type mice (Song et al., 2008).

During repetitive ORN stimulation designed to mimic sniffing, ORNs quite reliably generate APs to every stimulation when stimulated at a low (2 Hz; interpulse time, 400 ms) frequency at intermediate odorant concentrations. An increase to a stimulation rate more akin to sniffing (5 Hz; interpulse time, 100 ms) causes ORNs to fail to respond reliably with AP generation to every stimulation (Ghatpande and Reisert, 2011). At these higher frequencies, the interstimulus time is again too short and/or response termination mechanisms too slow to sufficiently terminate the receptor current to allow AP generation at the next stimulation. At high odorant concentrations, AP firing entirely stops during repetitive stimulation. This causes a reduction of information flow to the olfactory bulb from this particular ORN, an

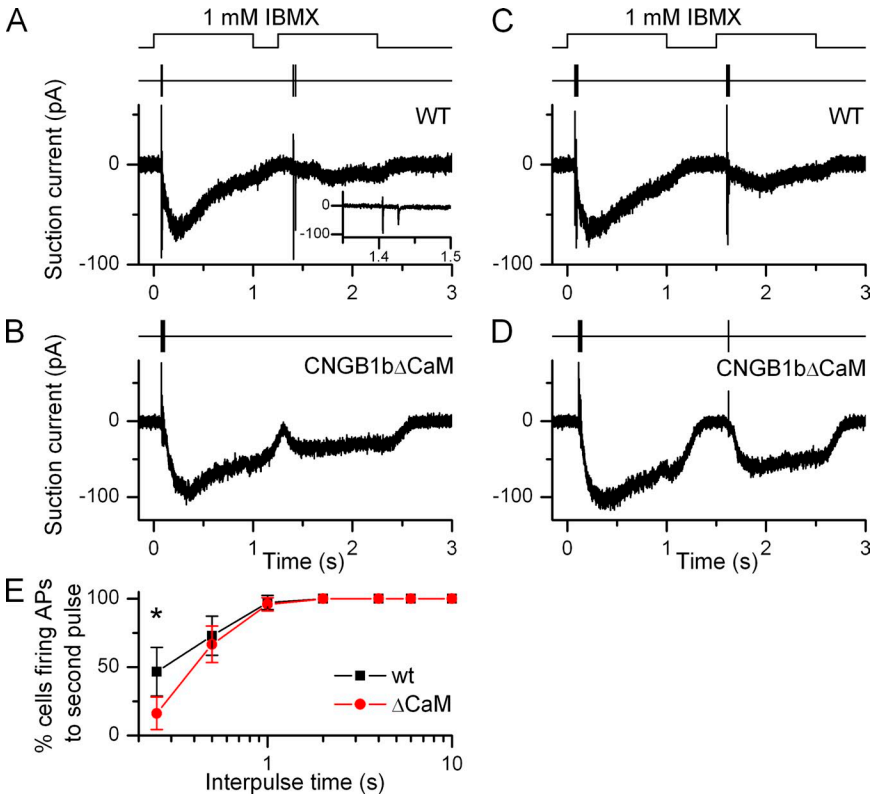

Figure 3. Fast response termination of the receptor current is required for reliable AP generation during repetitive stimulation. (A) Suction pipette recording from an isolated wild-type ORN. The ORN was exposed twice with an interpulse time of 0.25 s to the PDE inhibitor IBMX (see solution monitor at top). This led to an increase in ciliary cAMP and generation of the transduction current. APs were fired during the rising phases of both responses, as indicated by the AP raster plot above the current trace. The inset shows the rapid decline of the AP amplitude during the response. (B) The deletion of the CaM-binding site on the B1 subunit of the CNG channel ($CNGB1^{\Delta CaM}$) causes a subtle response prolongation, which reduces the possibility of APs being generated in response to the second IBMX exposure after a 0.25-s interpulse time. (C and D) The same ORNs as in A and B, respectively, but with the longer interpulse time of 0.5 s. Now both wild-type and $CNGB1^{\Delta CaM}$ ORN generate APs in response to the second stimulation. (E) Percentage of ORNs that fire APs in response to the second stimulation, dependent on the interpulse period. Data points are mean ± 95% confidence interval (n = 19–49 ORNs; *, χ^2 test; P = 0.007). Modified from Song et al. (2008) with permission from Elsevier.

effect that is mirrored by reduced presynaptic Ca^{2+} transients in the olfactory bulb during high frequency sniffing (Verhagen et al., 2007). Inhibitory responses, seen as a suppression of the basal spike rate, have also been reported but do not form the focus of this Perspective.

Rod photoreceptors are able to detect single photons because of the high amplification of their transduction cascade; a light-activated rhodopsin molecule stays active long enough to activate multiple G protein molecules. In contrast, ORNs lack this important amplification step, as an odorant molecule only stays bound to the OR for a very short time (Bhandawat et al., 2005), often too brief to activate even a single G protein. Thus, many OR activation events remain inconsequential. An activated OR does not, unlike rhodopsin in phototransduction, activate multiple G proteins, and an OR-binding event that does lead to G protein activation (successful odorant-binding event) only generates a small unitary current response of the order of 0.03 pA in frog ORNs (Bhandawat et al., 2010), too small to trigger APs. This raises the question of how many ORs have to be activated to reliably generate APs and signal to the olfactory bulb. Performing a series of experiments at low odorant concentrations and short exposure times, and equipped with the knowledge of the unitary response size, the chance of 50% AP generation per given odorant exposure was shown to require ∼40 successful odorant-binding events in frog ORNs (Bhandawat et al., 2010). The current required was estimated to be 1.2 pA. Broadly similar values have recently been shown for mouse ORNs (Ben-Chaim et al., 2011).

Mechanisms mediating adaptation?

During sustained odorant exposure ORNs generate a receptor current that increases to its peak and thereafter declines, often back to near zero current levels even before stimulus termination. Alternatively, adaptation is often studied in olfaction in a double-pulse paradigm, with the second response being progressively suppressed with shorter interpulse periods.

What are the mechanisms involved in such manifestations of adaptation? In particular, in light of the short lifetime of an activated OR, what mechanism shuts off an activated G protein? Although little is known about the lifetime of GTP-bound G_{olf} or the G_{olf}–ACIII complex, a putative guanine nucleotide exchange factor for G_{olf}, Ric-8b, has been identified in ORNs (Von Dannecker et al., 2006). A possible candidate to influence response kinetics early in the transduction cascade is olfactory marker protein (OMP). Mice lacking OMP have a greatly slowed response time course (Buiakova et al., 1996), and OMP modulation of the ciliary transduction cascade has been limited to ACIII and upstream transduction events, although the precise mechanism remains to be clarified. As a consequence of the slowed and delayed receptor current time course, OMP-knockout ORNs generate APs only after a longer delay of ∼170 ms after stimulation onset compared with 50 ms in wild-type ORNs. Also, the number of fired spikes increases from three in wild-type to five in OMP-knockout ORNs when stimulated with 100 µM cineole, probably because of the slowed rising phase of the receptor current in the mutant ORNs (Reisert et al., 2007).

Mechanisms involved in adaptation have been shown to be dependent on Ca^{2+} influx through the CNG channel, as chelating extracellular Ca^{2+} or positive holding potentials under whole cell voltage-clamped recording conditions greatly reduced the adaptive current decline (Kurahashi and Shibuya, 1990; Zufall et al., 1991; Boccaccio et al., 2006). A promising candidate for adaptation is the CNG channel itself (Zufall et al., 1991; Kurahashi and Menini, 1997). Indeed, Ca^{2+}, in the presence of calmodulin (CaM), reduces the channel's affinity to cAMP (Chen and Yau, 1994; Bradley et al., 2001). In a heterologously expressed CNG channel comprised only of the principle subunit CNGA2 (of the native heterotrimeric channel), a basic amphiphilic α-helix motif near the N terminus has been shown to mediate the Ca^{2+}-CaM–mediated desensitization (Liu et al., 1994). However, when all three olfactory CNG subunits, CNGA2 and the auxiliary CNGB1b and CNGA4, are expressed heterologously, two "IQ-type" CaM-binding sites on the N and C termini of the CNGB1b and CNGA4 subunits, respectively, mediate exogenous Ca^{2+}-CaM fast channel desensitization. CaM actually pre-associates with the CNG channel at basal Ca^{2+} levels (Bradley et al., 2001). Channels that lack either auxillary subunit inactivate much more slowly. Perplexingly, in a channel that still has its endogenous CaM bound, the IQ site on the CNGB1b subunit seems to be sufficient to mediate Ca^{2+}-CaM–mediated desensitization (Waldeck et al., 2009).

The role of Ca^{2+}-CaM–mediated CNG channel inhibition in adaptation was investigated in mice lacking either the CNGA4 or CNGB1b subunit. Indeed, ORNs from such mice showed odorant-induced responses that terminated much more slowly (Munger et al., 2001; Michalakis et al., 2006), and, when ORNs were stimulated twice in succession, a less reduced (less adapted) response to the second stimulation was recorded. This is consistent with the notion that, because of a lack of an auxillary CNG channel subunit, channel desensitization is slowed, causing a reduced adaptation of the odorant response. A confounder in this interpretation is that the knocking out of an entire auxiliary CNG subunit not only slows Ca^{2+}-CaM–mediated desensitization but also greatly reduces the overall sensitivity of the CNG channel to cAMP, and greatly reduces the ciliary channel density of the CNG channels due to reduced ciliary channel trafficking (Michalakis et al., 2006).

We chose a more limited and targeted genetic manipulation to address the involvement of CNG channel desensitization and odorant adaptation by generating a

mouse line that only lacked the IQ-binding site in the N terminus of the CNGB1b subunit (Song et al., 2008). In this CNGB1$^{\Delta CaM}$ mouse, neither the cAMP sensitivity of the CNG channel nor the ciliary channel density was changed. But as designed, Ca^{2+}-CaM–mediated CNG channel desensitization was slowed ~100-fold. Surprisingly, we did not see adaptational defects in receptor current generation as expected using a double-pulse stimulation protocol (see Fig. 3), suggesting that the role of Ca^{2+}-CaM–mediated adaptation on the CNG channel might not be as important as previously thought for ORN adaptation. Instead, as mentioned above, we observed a response prolongation of around 60 ms, which could arise from prolonged Ca^{2+} influx through the CNG channel as a result of reduced Ca^{2+}-CaM–mediated desensitization.

In addition to the CNG channel, ACIII has been suggested to be a feedback target of Ca^{2+} for adaptation, particularly adaptation that is induced by long odor exposures (Leinders-Zufall et al., 1999). Ca^{2+}, entering cilia through CNG channels, is thought to activate CaM-dependent kinase II (CaMKII), which then phosphorylates ACIII to reduce its activity and thus down-regulates cAMP production. This hypothesis was mainly derived from experiments conducted in heterologous expression systems (Wayman et al., 1995; Wei et al., 1996) and experiments using CaMKII inhibitors in olfactory cilia preparations and in isolated ORNs (Wei et al., 1998; Leinders-Zufall et al., 1999). Phosphorylation on a single serine residue, serine^{1076}, of ACIII was identified to account for Ca^{2+}/CaMKII-mediated inhibition (Wei et al., 1996, 1998). In a recent experiment (unpublished data), we again took a targeted genetic approach to address the potential involvement of this feedback mechanism in adaptation by generating a mouse line, which should lack CaMKII-mediated ACIII phosphorylation because of a single mutation of serine^{1076} to alanine. Surprisingly, electrophysiological recordings showed no differences in the responses between wild-type and mutant mice to single short (subsecond) or long (a few to tens of seconds) stimulations, or in several adaptation-stimulation paradigms. ORNs in the mutant mice show a similar slowing of the activation kinetics in responses after a prolonged stimulation as in wild-type mice, a phenomenon that is blocked by CaMKII inhibitors and has been suggested to be a result of inhibition of ACIII (Leinders-Zufall et al., 1999). The results in the mutant mice thus suggest that Ca^{2+}/CaMKII-mediated feedback inhibition of ACIII by phosphorylation is unlikely to play a significant role in adaptation.

Thus, the two historically main suspects for adaptational mechanisms, Ca^{2+}-CaM–negative feedback of the CNG channel via the IQ-binding site of the CNGB1b subunit and ACIII phosphorylation of serine^{1076}, are not playing their expected role, at least investigated individually. Currently, no other clear candidates for adaptation are waiting in the wings. Interestingly, Ca^{2+}-independent adaptation is also observed (Reisert et al., 2005), but its overall contribution to adaptation and its mechanism remains to be clarified.

Response termination

Termination of the response requires cAMP and Ca^{2+} levels to return to their pre-stimulus levels to allow the CNG and the Cl^- channel to close. Termination kinetics should be largely determined by whichever is slower, either the rate of cAMP removal or the rate of Ca^{2+} removal from cilia. In ORNs, cAMP is hydrolyzed by PDEs. So far, two PDEs, PDE1C and PDE4A, have been found to be expressed in ORNs. PDE1C is localized in the cilium, whereas PDE4A is present in the rest of the ORN including the dendritic knob, but not in the cilia (Juilfs et al., 1997). After being generated by ACIII in cilia upon odor stimulation, cAMP could theoretically either be degraded locally by PDE or diffuse into the dendrite, where it would be degraded by PDE4A. Although little is known about the fate of ciliary cAMP, recent knockout studies in mice revealed that removal of ciliary cAMP is not a rate-limiting factor for termination kinetics. Knockout of either PDE1C or PDE4A alone does not result in a prolonged termination phenotype (Cygnar and Zhao, 2009), suggesting that either PDE1C in the cilia or PDE4A outside the cilia is sufficient to allow rapid removal of ciliary cAMP and wild-type termination kinetics. PDE activity also does not contribute to fast adaptation during a double-pulse protocol (Boccaccio et al., 2006). A potential mechanism that could contribute to a ciliary cAMP reduction (and hence response termination) is diffusional escape of cAMP from the cilia into the cell body (Chen et al., 1999; Boccaccio et al., 2006; Flannery et al., 2006). This is supported by the observation that mice that lack both PDE1C and PDE4A do show slowed response termination (Cygnar and Zhao, 2009).

Accumulating evidence suggests that the rate of ciliary Ca^{2+} removal dictates the termination kinetics, and that Na^+/Ca^{2+} exchange is the major mechanism for ciliary Ca^{2+} removal in mice. Because ORN cilia lack intraciliary vesicular organelles, ciliary Ca^{2+} removal is thought to be achieved mainly by plasma membrane Ca^{2+} transporters including Na^+/Ca^{2+} exchangers and ATP-dependent Ca^{2+} pumps. The presence of Na^+/Ca^{2+} exchanger(s) in ORNs has been suggested since the mid-1990s. Preventing Ca^{2+} extrusion by reducing extracellular Na^+, and thus abolishing the driving gradient that fuels Na^+/Ca^{2+} exchange, prolongs the odorant-induced receptor current by seconds by generating a prolonged Ca^{2+}-activated Cl^- current (Reisert and Matthews, 2001; Antolin and Matthews, 2007). These results indicate that Na^+/Ca^{2+} exchange is critically important in mice for response termination. The very Na^+/Ca^{2+} exchanger(s) that is responsible for ciliary Ca^{2+} extrusion has not been determined (Pyrski et al., 2007). In a recent experiment,

we revealed that the potassium-dependent Na^+/Ca^{2+} exchanger 4 (NCKX4) is present in olfactory cilia preparations (Stephan et al., 2009) and that it likely functions for such purpose (Stephan et al., 2010). The NCKX4 knockout ORNs display prolonged response termination by up to several seconds, a phenotype mimicking what is seen in wild-type ORNs when replacing extracellular Na^+ to prevent Na^+-dependent Ca^{2+} extrusion. The lesser prolonged termination seen in NCKX4 heterozygous mice compared with the knockout supports that the rate of ciliary Ca^{2+} removal is the rate-limiting factor for the termination kinetics. It is interesting that NCKX4 knockout ORNs display normal response amplitude and activation kinetics to single-odorant pulses, suggesting that the basal level of ciliary Ca^{2+} is not greatly affected by the lack of NCKX4, maybe because during resting conditions, diffusional escape of ciliary Ca^{2+} in the dendrite is sufficient to keep ciliary Ca^{2+} levels low. But in a double-pulse stimulation paradigm, NCKX4 knockout ORNs show a severe reduction in the response to the second stimulus, much more pronounced than that seen in $CNGB1^{\Delta CaM}$ ORNs. Consequently, the ability of AP generation to repeated stimulation is also greatly reduced, emphasizing the importance of NCKX4 in rapid response termination in olfactory coding during repeated stimulation.

Response and AP generation in vivo

Immense progress has been made over recent years in understanding odorant responses in the olfactory bulb, using either imaging or electrophysiological approaches in the behaving mouse or rat. But little is known about how mammalian ORNs respond in the live animal during natural breathing. A successful approach to start addressing this problem has been to record AP extracellularly in anesthetized rats (Duchamp-Viret et al., 1999) using sharp electrodes inserted through a small hole in the nasal bone, with odorant application so far being restricted to long (e.g., 2-s) odorant exposures directly to the olfactory epithelium. Alternatively, the electroolfactogram, which records the ensemble activity of ORNs, can be recorded from medial or lateral turbinates, with electrodes inserted through holes in the nasal bones (Scott-Johnson et al., 2000) in an anesthetized, artificially ventilated rat. An important issue to consider in these recording configurations is the unique (probably with the exception of taste receptors in the mouth) environment ORNs have to function in. Although the cell bodies reside within the olfactory epithelium in a normal ionic environment, cilia, where transduction takes place, have to function outside of the body in the environment of the olfactory mucus. The ion concentrations in mucus are significantly different compared with interstitial fluid and might also change, especially in amphibians. The few measurements to determine the mucosal ion concentrations suggest that Cl^- might be as low as 50 mM (Chiu et al., 1988; Reuter et al., 1998), a concentration that would considerably increase the excitatory Cl^- current by facilitating Cl^- efflux. Also, mucosal Na^+ is low (Chiu et al., 1988; Reuter et al., 1998) and close to the K_d of the (amphibian) $Na^+/Ca^{2+}/(K^+?)$ exchanger (Antolin and Matthews, 2007). This could begin to slow response recovery due to slowed Ca^{2+} removal from the cilia. Indeed, at least in isolated ORNs, preventing or slowing Ca^{2+} extrusion can entirely suppress AP generation during repetitive stimulation (Reisert and Matthews, 1998), even at stimulation frequencies as low as 2 Hz (Ghatpande and Reisert, 2011). This slowed Ca^{2+} removal can further limit ORNs to respond faithfully to rapid stimulation.

So how many sniff cycles are required to determine the identity of an odorant? Well-trained mice and rats can distinguish odorant in <200 ms (Uchida and Mainen, 2003; Abraham et al., 2004; Rinberg et al., 2006), the equivalent of a single sniff at 5 Hz. But the odorant sampling duration progressively lengthens to up to 500 ms when mice have to perform more complex odorant discrimination tasks (Rinberg et al., 2006), suggesting that under such conditions, repeated odorant exposures might be required. Thus, unraveling how ORNs encode for odorants during repeated stimulations at breathing rates at rest or during high frequency sniff bouts in a freely breathing animal will be an important question to understand and interpret the olfactory code. As will understanding how each of the aspects of the olfactory response (e.g., timing, frequency, number of APs, synchronicity across ORNs targeting the same glomeruli, etc.) contributes specifically to the olfactory code.

This Perspectives series includes articles by Farley and Sampath, Schwartz and Rieke, Bautista and Lumpkin, and Zhang et al.

The authors would like to thank Drs. C.-Y. Su and G. Lowe for critical and constructive reading of the manuscript.

This work was supported by National Institutes of Health grants DC007395 (to H. Zhao), DC009613 (to J. Reisert), and DC009946 (to H. Zhao and J. Reisert), and a Morley Kare Fellowship (to J. Reisert).

Robert S. Farley served as guest editor.

REFERENCES

Abraham, N.M., H. Spors, A. Carleton, T.W. Margrie, T. Kuner, and A.T. Schaefer. 2004. Maintaining accuracy at the expense of speed: stimulus similarity defines odor discrimination time in mice. *Neuron.* 44:865–876.

Antolin, S., and H.R. Matthews. 2007. The effect of external sodium concentration on sodium-calcium exchange in frog olfactory receptor cells. *J. Physiol.* 581:495–503. doi:10.1113/jphysiol.2007.131094

Bautista, D.M., and E.A. Lumpkin. 2011. Probing mammalian touch transduction. *J. Gen. Physiol.* 138:291–301.

Ben-Chaim, Y., M.M. Cheng, and K.W. Yau. 2011. Unitary response of mouse olfactory receptor neurons. *Proc. Natl. Acad. Sci. USA.* 108:822–827. doi:10.1073/pnas.1017983108

Bhandawat, V., J. Reisert, and K.W. Yau. 2005. Elementary response of olfactory receptor neurons to odorants. *Science.* 308:1931–1934. doi:10.1126/science.1109886

Bhandawat, V., J. Reisert, and K.W. Yau. 2010. Signaling by olfactory receptor neurons near threshold. *Proc. Natl. Acad. Sci. USA.* 107:18682–18687. doi:10.1073/pnas.1004571107

Billig, G.M., B. Pál, P. Fidzinski, and T.J. Jentsch. 2011. Ca^{2+}-activated Cl^- currents are dispensable for olfaction. *Nat. Neurosci.* 14:763–769. doi:10.1038/nn.2821

Boccaccio, A., and A. Menini. 2007. Temporal development of cyclic nucleotide-gated and Ca^{2+}-activated Cl^- currents in isolated mouse olfactory sensory neurons. *J. Neurophysiol.* 98:153–160. doi:10.1152/jn.00270.2007

Boccaccio, A., L. Lagostena, V. Hagen, and A. Menini. 2006. Fast adaptation in mouse olfactory sensory neurons does not require the activity of phosphodiesterase. *J. Gen. Physiol.* 128:171–184. doi:10.1085/jgp.200609555

Bradley, J., D. Reuter, and S. Frings. 2001. Facilitation of calmodulin-mediated odor adaptation by cAMP-gated channel subunits. *Science.* 294:2176–2178. doi:10.1126/science.1063415

Buiakova, O.I., H. Baker, J.W. Scott, A. Farbman, R. Kream, M. Grillo, L. Franzen, M. Richman, L.M. Davis, S. Abbondanzo, et al. 1996. Olfactory marker protein (OMP) gene deletion causes altered physiological activity of olfactory sensory neurons. *Proc. Natl. Acad. Sci. USA.* 93:9858–9863. doi:10.1073/pnas.93.18.9858

Chen, C.H., T. Nakamura, and Y. Koutalos. 1999. Cyclic AMP diffusion coefficient in frog olfactory cilia. *Biophys. J.* 76:2861–2867. doi:10.1016/S0006-3495(99)77440-0

Chen, T.-Y., and K.-W. Yau. 1994. Direct modulation by Ca^{2+}-calmodulin of cyclic nucleotide-activated channel of rat olfactory receptor neurons. *Nature.* 368:545–548. doi:10.1038/368545a0

Chiu, D., T. Nakamura, and G.H. Gold. 1988. Ionic composition of toad olfactory mucus measured with ion-selective microelectrodes. *Chem. Senses.* 13:677–678.

Cygnar, K.D., and H. Zhao. 2009. Phosphodiesterase 1C is dispensable for rapid response termination of olfactory sensory neurons. *Nat. Neurosci.* 12:454–462. doi:10.1038/nn.2289

Duchamp-Viret, P., M.A. Chaput, and A. Duchamp. 1999. Odor response properties of rat olfactory receptor neurons. *Science.* 284:2171–2174. doi:10.1126/science.284.5423.2171

Flannery, R.J., D.A. French, and S.J. Kleene. 2006. Clustering of cyclic-nucleotide-gated channels in olfactory cilia. *Biophys. J.* 91:179–188. doi:10.1529/biophysj.105.079046

Gesteland, R.C., and C.D. Sigwart. 1977. Olfactory receptor units—a mammalian preparation. *Brain Res.* 133:144–149. doi:10.1016/0006-8993(77)90055-5

Ghatpande, A.S., and J. Reisert. 2011. Olfactory receptor neuron responses coding for rapid odour sampling. *J. Physiol.* 589:2261–2273. doi:10.1113/jphysiol.2010.203687

Hengl, T., H. Kaneko, K. Dauner, K. Vocke, S. Frings, and F. Möhrlen. 2010. Molecular components of signal amplification in olfactory sensory cilia. *Proc. Natl. Acad. Sci. USA.* 107:6052–6057. doi:10.1073/pnas.0909032107

Juilfs, D.M., H.J. Fülle, A.Z. Zhao, M.D. Houslay, D.L. Garbers, and J.A. Beavo. 1997. A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. *Proc. Natl. Acad. Sci. USA.* 94:3388–3395. doi:10.1073/pnas.94.7.3388

Kawai, F., T. Kurahashi, and A. Kaneko. 1997. Quantitative analysis of Na^+ and Ca^{2+} current contributions on spike initiation in the newt olfactory receptor cell. *Jpn. J. Physiol.* 47:367–376. doi:10.2170/jjphysiol.47.367

Kepecs, A., N. Uchida, and Z.F. Mainen. 2007. Rapid and precise control of sniffing during olfactory discrimination in rats. *J. Neurophysiol.* 98:205–213. doi:10.1152/jn.00071.2007

Kleene, S.J. 2008. The electrochemical basis of odor transduction in vertebrate olfactory cilia. *Chem. Senses.* 33:839–859. doi:10.1093/chemse/bjn048

Kurahashi, T., and A. Menini. 1997. Mechanism of odorant adaptation in the olfactory receptor cell. *Nature.* 385:725–729. doi:10.1038/385725a0

Kurahashi, T., and T. Shibuya. 1990. Ca^{2+}-dependent adaptive properties in the solitary olfactory receptor cell of the newt. *Brain Res.* 515:261–268. doi:10.1016/0006-8993(90)90605-B

Leinders-Zufall, T., M. Ma, and F. Zufall. 1999. Impaired odor adaptation in olfactory receptor neurons after inhibition of Ca^{2+}/calmodulin kinase II. *J. Neurosci.* 19:RC19.

Liu, M.Y., T.Y. Chen, B. Ahamed, J. Li, and K.-W. Yau. 1994. Calcium-calmodulin modulation of the olfactory cyclic nucleotide-gated cation channel. *Science.* 266:1348–1354. doi:10.1126/science.266.5189.1348

Lowe, G., and G.H. Gold. 1993. Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells. *Nature.* 366:283–286. doi:10.1038/366283a0

Michalakis, S., J. Reisert, H. Geiger, C. Wetzel, X. Zong, J. Bradley, M. Spehr, S. Hüttl, A. Gerstner, A. Pfeifer, et al. 2006. Loss of CNGB1 protein leads to olfactory dysfunction and subciliary cyclic nucleotide-gated channel trapping. *J. Biol. Chem.* 281:35156–35166. doi:10.1074/jbc.M606409200

Munger, S.D., A.P. Lane, H. Zhong, T. Leinders-Zufall, K.W. Yau, F. Zufall, and R.R. Reed. 2001. Central role of the CNGA4 channel subunit in Ca^{2+}-calmodulin-dependent odor adaptation. *Science.* 294:2172–2175. doi:10.1126/science.1063224

Pyrski, M., J.H. Koo, S.K. Polumuri, A.M. Ruknudin, J.W. Margolis, D.H. Schulze, and F.L. Margolis. 2007. Sodium/calcium exchanger expression in the mouse and rat olfactory systems. *J. Comp. Neurol.* 501:944–958. doi:10.1002/cne.21290

Rasche, S., B. Toetter, J. Adler, A. Tschapek, J.F. Doerner, S. Kurtenbach, H. Hatt, H. Meyer, B. Warscheid, and E.M. Neuhaus. 2010. Tmem16b is specifically expressed in the cilia of olfactory sensory neurons. *Chem. Senses.* 35:239–245. doi:10.1093/chemse/bjq007

Reisert, J., and H.R. Matthews. 1998. Na^+-dependent Ca^{2+} extrusion governs response recovery in frog olfactory receptor cells. *J. Gen. Physiol.* 112:529–535. doi:10.1085/jgp.112.5.529

Reisert, J., and H.R. Matthews. 1999. Adaptation of the odour-induced response in frog olfactory receptor cells. *J. Physiol.* 519:801–813. doi:10.1111/j.1469-7793.1999.0801n.x

Reisert, J., and H.R. Matthews. 2001. Response properties of isolated mouse olfactory receptor cells. *J. Physiol.* 530:113–122. doi:10.1111/j.1469-7793.2001.0113m.x

Reisert, J., and D. Restrepo. 2009. Molecular tuning of odorant receptors and its implication for odor signal processing. *Chem. Senses.* 34:535–545. doi:10.1093/chemse/bjp028

Reisert, J., J. Lai, K.W. Yau, and J. Bradley. 2005. Mechanism of the excitatory Cl^- response in mouse olfactory receptor neurons. *Neuron.* 45:553–561. doi:10.1016/j.neuron.2005.01.012

Reisert, J., K.W. Yau, and F.L. Margolis. 2007. Olfactory marker protein modulates the cAMP kinetics of the odour-induced response in cilia of mouse olfactory receptor neurons. *J. Physiol.* 585:731–740. doi:10.1113/jphysiol.2007.142471

Reuter, D., K. Zierold, W.H. Schröder, and S. Frings. 1998. A depolarizing chloride current contributes to chemoelectrical transduction in olfactory sensory neurons in situ. *J. Neurosci.* 18:6623–6630.

Rinberg, D., A. Koulakov, and A. Gelperin. 2006. Speed-accuracy tradeoff in olfaction. *Neuron.* 51:351–358. doi:10.1016/j.neuron.2006.07.013

Rospars, J.P., P. Lansky, M. Chaput, and P. Duchamp-Viret. 2008. Competitive and noncompetitive odorant interactions in the early neural coding of odorant mixtures. *J. Neurosci.* 28:2659–2666. doi:10.1523/JNEUROSCI.4670-07.2008

Schwartz, G., and F. Rieke. 2011. Nonlinear spatial encoding by retinal ganglion cells: when 1 + 1 ≠ 2. *J. Gen. Physiol.* 138:283–290.

Scott-Johnson, P.E., D. Blakley, and J.W. Scott. 2000. Effects of air flow on rat electroolfactogram. *Chem. Senses.* 25:761–768. doi:10.1093/chemse/25.6.761

Shibuya, T., and S. Shibuya. 1963. Olfactory epithelium: unitary responses in the tortoise. *Science.* 140:495–496. doi:10.1126/science.140.3566.495

Song, Y., K.D. Cygnar, B. Sagdullaev, M. Valley, S. Hirsh, A. Stephan, J. Reisert, and H. Zhao. 2008. Olfactory CNG channel desensitization by Ca^{2+}/CaM via the B1b subunit affects response termination but not sensitivity to recurring stimulation. *Neuron.* 58:374–386. doi:10.1016/j.neuron.2008.02.029

Stephan, A.B., E.Y. Shum, S. Hirsh, K.D. Cygnar, J. Reisert, and H. Zhao. 2009. ANO2 is the cilial calcium-activated chloride channel that may mediate olfactory amplification. *Proc. Natl. Acad. Sci. USA.* 106:11776–11781. doi:10.1073/pnas.0903304106

Stephan, A.B., S. Tobochnik, J. Reisert, and H. Zhao. 2010. NCKX4, a calcium regulator, efficiently terminates the olfactory response and moderates the extent of adaptation. *Chem. Senses.* 31:A17.

Su, C.Y., K. Menuz, and J.R. Carlson. 2009. Olfactory perception: receptors, cells, and circuits. *Cell.* 139:45–59. doi:10.1016/j.cell.2009.09.015

Tankersley, C.G., R.S. Fitzgerald, and S.R. Kleeberger. 1994. Differential control of ventilation among inbred strains of mice. *Am. J. Physiol.* 267:R1371–R1377.

Trotier, D. 1994. Intensity coding in olfactory receptor cells. *Semin. Cell Biol.* 5:47–54. doi:10.1006/scel.1994.1007

Uchida, N., and Z.F. Mainen. 2003. Speed and accuracy of olfactory discrimination in the rat. *Nat. Neurosci.* 6:1224–1229. doi:10.1038/nn1142

Verhagen, J.V., D.W. Wesson, T.I. Netoff, J.A. White, and M. Wachowiak. 2007. Sniffing controls an adaptive filter of sensory input to the olfactory bulb. *Nat. Neurosci.* 10:631–639. doi:10.1038/nn1892

Von Dannecker, L.E., A.F. Mercadante, and B. Malnic. 2006. Ric-8B promotes functional expression of odorant receptors. *Proc. Natl. Acad. Sci. USA.* 103:9310–9314. doi:10.1073/pnas.0600697103

Waldeck, C., K. Vocke, N. Ungerer, S. Frings, and F. Möhrlen. 2009. Activation and desensitization of the olfactory cAMP-gated transduction channel: identification of functional modules. *J. Gen. Physiol.* 134:397–408. doi:10.1085/jgp.200910296

Wayman, G.A., S. Impey, and D.R. Storm. 1995. Ca^{2+} inhibition of type III adenylyl cyclase *in vivo*. *J. Biol. Chem.* 270:21480–21486. doi:10.1074/jbc.270.37.21480

Wei, J., G. Wayman, and D.R. Storm. 1996. Phosphorylation and inhibition of type III adenylyl cyclase by calmodulin-dependent protein kinase II *in vivo*. *J. Biol. Chem.* 271:24231–24235. doi:10.1074/jbc.271.39.24231

Wei, J., A.Z. Zhao, G.C.K. Chan, L.P. Baker, S. Impey, J.A. Beavo, and D.R. Storm. 1998. Phosphorylation and inhibition of olfactory adenylyl cyclase by CaM kinase II in neurons: a mechanism for attenuation of olfactory signals. *Neuron.* 21:495–504. doi:10.1016/S0896-6273(00)80561-9

Youngentob, S.L., M.M. Mozell, P.R. Sheehe, and D.E. Hornung. 1987. A quantitative analysis of sniffing strategies in rats performing odor detection tasks. *Physiol. Behav.* 41:59–69. doi:10.1016/0031-9384(87)90131-4

Zufall, F., G.M. Shepherd, and S. Firestein. 1991. Inhibition of the olfactory cyclic nucleotide gated ion channel by intracellular calcium. *Proc. Biol. Sci.* 246:225–230. doi:10.1098/rspb.1991.0148