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Elementary Response of Olfactory Receptor Neurons to Odorants

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Abstract

Signaling by heterotrimeric GTP-binding proteins (G proteins) drives numerous cellular processes. The number of G protein molecules activated by a single membrane receptor is a determinant of signal amplification, although in most cases this parameter remains unknown. In retinal rod photoreceptors, a long-lived photoisomerized rhodopsin molecule activates many G protein molecules (transducins), yielding substantial amplification and a large elementary (single-photon) response, before rhodopsin activity is terminated. Here we report that the elementary response in olfactory transduction is extremely small. A ligand-bound odorant receptor has a low probability of activating even one G protein molecule because the odorant dwell-time is very brief. Thus, signal amplification in olfactory transduction appears fundamentally different from that of phototransduction.

Odorants activate specific receptor proteins (1) on the cilia of olfactory receptor neurons (ORNs) and, by way of a G protein (G_{olf}), stimulate an adenylyl cyclase (type III) to synthesize adenosine 3',5'-cyclic monophosphate (cAMP) (2,3). cAMP opens a cyclic-nucleotide-gated (CNG) cation channel to produce a membrane depolarization (2,3). Influx of Ca^{2+} through the CNG channel opens a Ca^{2+} -activated chloride (Cl) channel, leading to Cl^- efflux and further depolarization (2,3). Simultaneously, the Ca^{2+} influx decreases cAMP synthesis and the effective affinity of CNG channels for cAMP, both effects producing olfactory adaptation (2, 3).

Little is known about signal amplification in olfactory transduction. It has been suggested (4) that, in physiological (Ringer) solution, a single odorant-receptor molecule triggers an elementary (or unitary) olfactory response of ~ 1 pA in membrane current, indicating an amplification similar to that in phototransduction. However, this conclusion has been challenged (5,6). The supralinear relation (i.e., Hill coefficient > 1) reported between odorant concentration and response amplitude (7) is also puzzling because it may suggest a nonlinear summation of the elementary responses. At odorant concentrations low enough to give few odorant-binding events, the overall response should arise from spatially segregated, noninteracting transduction domains on the cilia triggered by individual activated membrane receptor molecules (the "units"). Thus, despite intrinsic transduction nonlinearities [multiple cAMP molecules are required to open a CNG channel (2,3) and multiple Ca^{2+} to open a

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Supporting Online Material

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Materials and Methods

Figs. S1 to S4

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Ca²⁺-activated Cl channel (2,3)], these segregated domains should sum linearly, as is the case for single-photon responses in rod photoreceptors (8,9).

To characterize the elementary olfactory response, we measured membrane currents from single, dissociated frog ORNs with the suction-pipette method (10,11). By stimulating an ORN in normal Ringer solution with a brief pulse of the odorant cineole (12), we confirmed a supralinear relation between response amplitude and odorant concentration (Hill coefficient $n = 1.5$ to 6.0 ; mean $\pm$ SD = 3.0 ± 1.6 from nine cells) (Fig. 1, A and C). At low ($20 \mu\text{M}$) external Ca²⁺ concentration [replaced by equimolar Mg²⁺ to retain divalent block of the CNG channel (13)], the response to a weak stimulus increased substantially, presumably owing to removal of Ca²⁺-dependent adaptation. The foot of the dose-response relation also became linear (Fig. 1, B to D) (14 cells). Likewise, a supralinear relation between response amplitude and odorant duration (at constant concentration) in Ringer solution (Fig. 1E) ($n = 2.8 \pm 0.8$ from six cells) became linear in $20 \mu\text{M}$ Ca²⁺ solution (Fig. 1F) (14 cells). The simplest interpretation of the linearity is that only one odorant molecule is required for activating a membrane receptor and that, at low event frequencies, the elementary responses indeed sum linearly. The odorant concentrations in Fig. 1, A to F, were high because of the short odorant pulses used. Longer stimulus durations in either Ringer or $20 \mu\text{M}$ Ca²⁺ solution decreased the half-saturating odorant concentration ($K_{1/2}$) of the dose-response relation (Fig. 1, G and H). The lowest $K_{1/2}$ with a 500-ms cineole stimulus was $\sim 1 \mu\text{M}$ from more than 340 cineole-responsive cells (12); this value would presumably be even lower with longer stimulus durations.

To perform quantal analysis (14) for extracting information about the unitary response, we decreased the external Ca²⁺ concentration to 100 nM to further increase the response. Successive weak, identical odorant pulses elicited responses with a constant time course but fluctuating, quantized amplitudes (Fig. 2, A and B). Assuming Poisson statistics, the variance/mean ratio (σ^2/m) of the response ensemble (8) (Fig. 2B, inset) gave a unitary response amplitude of 0.9 pA , matching the first nonzero peak in the amplitude histogram. Dividing the mean response (2.9 pA) by 0.9 pA yielded a mean quantal content of 3.2 . The predicted amplitude distribution can thus be generated from the Poisson distribution (Fig. 2B) (12). This fits well with the experimental histogram, hence validating Poisson statistics. A total of five cells were analyzed, with similar results. The unitary amplitude was quite similar from cell to cell (Fig. 2C) ($0.94 \pm 0.19 \text{ pA}$; 19 cells, including 14 with only σ^2/m values).

The quantal analysis was repeated with an external Ca²⁺ concentration of $20 \mu\text{M}$. The unitary response was smaller (0.42 pA in Fig. 3, A to C) and only extractable from σ^2/m . In cases (e.g., Fig. 3D) where the unitary amplitude was estimated at several mean response amplitudes (by varying odorant concentration or duration) in the same cell, this value was fairly constant, further validating the variance analysis. Again, the unit across cells was quite constant ($0.40 \pm 0.07 \text{ pA}$, 18 cells) (Fig. 3E), despite randomly selected ORNs [each of which should have a different odorant receptor (1, 15, 16)] and the use of several odorants (cineole, isoamylacetate, and acetophenone). Thus, the unitary response amplitude appears to be independent of the odorant or the receptor.

As expected, the quantal analysis in normal Ringer solution failed, owing to the nonlinear dose-response relation. Nonetheless, the unitary amplitude can still be estimated. Linear extrapolation from the foot of the dose-response relation in Ringer solution containing $20 \mu\text{M}$ Ca²⁺ (Fig. 1C) gave a macroscopic current of 132 pA at $300 \mu\text{M}$ cineole. Dividing this value by a unitary amplitude of 0.4 pA at this Ca²⁺ concentration yields 330 events. In Ringer solution, the same cineole concentration elicited a response of only 5 pA . Thus, the unitary amplitude in Ringer solution would be $5/330 = 0.015 \text{ pA}$ (assuming the receptor-odorant interaction to be Ca²⁺ independent). This is an upper estimate because some nonlinear

summation of units may already exist at 5 pA. From analysis of five cells, similar calculations gave a mean unit size of $0.026 (\pm 0.015)$ pA, a factor of 100 smaller than previously reported (4). Why is the foot of the dose-response relation linear in low- Ca^{2+} solution but not in Ringer solution? Simply, the unitary response in Ringer solution is so small that, in any detectable macroscopic response, there are already so many binding events that their domains overlap spatially and hence sum supralinearly owing to the intrinsic transduction nonlinearities.

To confirm that the unitary response is independent of the receptor-odorant complex (Figs. 2C and 3E), we examined ORNs that responded to two odorants separately (very rare encounters). In Fig. 4A, a 50-ms pulse of either 1 or 2 mM acetophenone in $20 \mu\text{M Ca}^{2+}$ solution elicited small, identical responses (suggesting that all receptors were bound). In contrast, a 1 mM cineole pulse half as long (25 ms) produced a response seven times as large as that to acetophenone, possibly before saturating all receptors. Thus, the efficacy of cineole in activating the odorant receptor in this cell was at least 14 times that of acetophenone. Nonetheless, the unitary responses derived from σ^2/m were ~ 0.5 pA for both odorants and had comparable response kinetics (Fig. 4A). Four other cells gave similar results.

The similar response kinetics elicited by two odorants of widely different efficacies on a cell suggests that the odorant dwell-time [a parameter coupled to the efficacy of the receptor-odorant complex (12)] is not a dominant time constant in the response waveform; otherwise, the more effective odorant would have elicited a more prolonged response (12). Also, if a single membrane receptor, during the odorant dwell-time, activates a large number of G protein molecules, an odorant with a longer dwell-time should produce a larger unitary response. Thus, a parsimonious interpretation of the constant unitary response is that the receptor-odorant complex has a low probability of activating even one G_{olf} molecule (although this probability will still determine the relative sizes of the macroscopic responses triggered by different receptor-odorant complexes). Consequently, the action of one G_{olf} molecule [formally equivalent to the action of one adenylyl cyclase molecule because one G protein molecule at most activates one adenylyl cyclase molecule (17)] should become the dominant unitary event underlying the stochastic response fluctuations. This could explain the constancy of the unitary response because essentially all ORNs use G_{olf} and adenylyl cyclase for transduction. In short, a very low amplification exists between an odorant-binding event and the activation of adenylyl cyclase. Theoretically, an alternative scenario of one receptor activating multiple G_{olf} molecules is also possible, but the probability of G_{olf} activating adenylyl cyclase would have to be proportionately reduced further. We think this scenario is unlikely because of the short dwell-time of odorant on the receptor (see below).

Not only is the relation between response amplitude and odorant duration linear in low- Ca^{2+} solution, but it also has a time intercept near zero (14 cells) (Fig. 1F). This time intercept is a measure of the effective odorant dwell-time, provided the odorant on-rate far exceeds its off-rate (12). Accordingly, we stimulated an ORN (at $20 \mu\text{M Ca}^{2+}$) with an odorant pulse at concentrations high enough to bind all of the receptors. Twenty-five millisecond pulses of cineole at 1, 1.5, or 2 mM all produced the same response amplitude (Fig. 4B), indicating saturation of the receptors. When applied for 50 ms at these concentrations, the pulses produced responses exactly twice as large, indicating that the responses were within the linear range (i.e., no compression due to downstream transduction steps) with respect to their dependence on odorant duration. The relation between odorant duration and response amplitude at 2 mM cineole extrapolated to a time intercept near zero (Fig. 4B). A total of six experiments gave a time intercept of -3.2 to $+8.1$ ms (mean $\pm$ SD = $+2.3 \pm 4.1$ ms; the small positive mean value perhaps reflected slight measurement uncertainties). Thus, the odorant dwell-time on the receptor was at most on the order of 1 ms. Because it is so short-lived, the receptor-odorant complex is unlikely to activate a G_{olf} molecule. Even for rod phototransduction, known for its high amplification, one photoisomerized rhodopsin molecule will activate only 0.1 G protein

(transducin) molecule in 1 ms (12,18). Indeed, even when rendered continuously bound to ligand by high odorant concentration (Fig. 4B), the receptor apparently still had a low probability of activating any G_{olf} in a time window up to 50 ms (up to at least 100 ms in other experiments); otherwise, concatenated binding events on the same receptor molecule would have produced overlapping domains of activation and a nonlinear relation between macroscopic response and odorant duration. In short, the brief odorant dwell-time leads to a low probability of activating G_{olf} , consistent with the constancy of the unitary event across cells. This interpretation does not depend on the detailed molecular mechanism for the receptor- G_{olf} interaction, either by diffusion (as with rhodopsin-transducin interactions) or by close-range interactions in a complex of signaling molecules. If a signaling complex exists, its purpose is unclear because of the low probability that the receptor-odorant complex will activate G_{olf} .

It is generally thought that one active G protein-coupled receptor (GPCR) molecule activates multiple downstream G protein/effector enzyme molecules, providing amplification. In rod phototransduction, one photoisomerized rhodopsin certainly activates many transducins before shutoff by phosphorylation and arrestin binding (18,19). We find that this is not necessarily so for olfactory transduction (and, by extension, perhaps some other ligand-activated GPCR pathways as well). The receptor-odorant complexes, at least those observed here, lasted <1 ms. Apart from a low probability of activating any G_{olf} , the complexes may be too short-lived to be phosphorylated by a G protein-coupled receptor kinase (GRK), whether or not this mechanism exists in ORNs (20–22). Our experiments indicate that, even if continuously bound to ligand, a receptor is not inactivated at least up to the order of 100 ms (otherwise the stimulus duration-response relation in Fig. 4B would not have remained linear). Thus, phosphorylation and arrestin binding are unlikely to constitute the standard termination of olfactory responses. Possibly, phosphorylation is important for desensitization in situations of prolonged and intense stimulation.

A short-lived receptor-odorant complex does not preclude an overall high olfactory sensitivity. Repeated bindings of odorant molecules to the same receptor allow signal integration, especially if receptor phosphorylation does not occur (unlike in vision, where a photon acts only once and a photobleached pigment molecule is nonfunctional). The total rate of odorant-binding events is also amplified by orders of magnitude by the total number of receptor molecules on an ORN. The supralinear interactions occurring when unitary transduction domains overlap can further enhance sensitivity at intermediate odorant concentrations and durations. Finally, a high convergence of sensory input at the glomerulus (23) may boost sensitivity. The glomerulus is the synaptic plexus in the olfactory bulb that integrates signals from all ORNs expressing the same odorant receptor species. In principle, this convergence can increase indefinitely by simply expanding the surface area of the olfactory epithelium and therefore the number of ORNs expressing a given odorant receptor. This increase in convergence may explain why the olfactory sensitivity in many animals is much higher than it is in humans. Unlike the retinotopic map in vision, which imposes a functional limit on the degree of convergence from photoreceptors, no corresponding limitation exists in olfaction.

Supplementary Material

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References and Notes

1. Buck LB. Cell 2000;100:611. [PubMed: 10761927]
2. Schild D, Restrepo D. Physiol. Rev 1998;78:429. [PubMed: 9562035]
3. Matthews HR, Reisert J. Curr. Opin. Neurobiol 2003;13:469. [PubMed: 12965295]

4. Menini A, Picco C, Firestein S. *Nature* 1995;373:435. [PubMed: 7830795]
5. Lowe G, Gold GH. *Proc. Natl. Acad. Sci. U.S.A* 1995;92:7864. [PubMed: 7544007]
6. Gold GH, Lowe G. *Nature* 1995;376:27. [PubMed: 7646719]
7. Firestein S, Picco C, Menini A. *J. Physiol* 1993;468:1. [PubMed: 8254501]
8. Baylor DA, Lamb TD, Yau K-W. *J. Physiol* 1979;288:613. [PubMed: 112243]
9. Lamb TD, McNaughton PA, Yau K-W. *J. Physiol* 1981;319:463. [PubMed: 6798202]
10. Lowe G, Gold GH. *J. Physiol* 1991;442:147. [PubMed: 1798028]
11. Reisert J, Matthews HR. *J. Gen. Physiol* 1998;112:529. [PubMed: 9806962]
12. Materials and methods as well as additional notes are available as supporting material on *Science* Online.
13. Kleene SJ. *Neuroscience* 1995;66:1001. [PubMed: 7651604]
14. del Castillo J, Katz B. *J. Physiol* 1954;124:560. [PubMed: 13175199]
15. Serizawa S, et al. *Science* 2003;302:2088. [PubMed: 14593185]
16. Lewcock JW, Reed RR. *Proc. Natl. Acad. Sci. U.S.A* 2004;101:1069. [PubMed: 14732684]
17. Tesmer JJ, Sunahara RK, Gilman AG, Sprang SR. *Science* 1997;278:1907. [PubMed: 9417641]
18. Leskov, et al. *Neuron* 2000;27:525. [PubMed: 11055435]
19. Yau K-W. *Invest. Ophthalmol. Vis. Sci* 1994;35:9. [PubMed: 7507907]
20. Dawson TM, et al. *Science* 1993;259:825. [PubMed: 8381559]
21. Schleicher S, Boekhoff I, Arriza J, Lefkowitz RJ, Breer H. *Proc. Natl. Acad. Sci. U.S.A* 1993;90:1420. [PubMed: 8381966]
22. Peppel K, et al. *J. Biol. Chem* 1997;272:25425. [PubMed: 9325250]
23. Mombaerts P, et al. *Cell* 1996;87:675. [PubMed: 8929536]
24. Nakatani K, Yau K-W. *Nature* 1988;334:69. [PubMed: 3386743]
25. Nakatani K, Yau K-W. *J. Physiol* 1988;395:695. [PubMed: 2457685]
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Fig. 1.

Odorant-induced responses of an isolated frog ORN in normal and low (20 μM)– Ca^{2+} Ringer solutions. (A to C) Comparison of responses from the same cell in normal and low- Ca^{2+} Ringer solutions. (A) Normal Ringer solution. Responses to a 25-ms pulse of cineole at 300, 500, 1000, and 2000 μM , respectively. Each trace represents the average of two to five stimulus trials (five for each of the two smallest responses). (B) Low- Ca^{2+} Ringer solution. Responses to a 25-ms pulse of cineole at 50, 100, 300, 500, 1000, and 1500 μM , respectively. Each trace represents the average of two to five trials. (C) The dependence of the transient peak current on odorant concentration in (A) and (B) are plotted for comparison. The smooth curve for normal Ringer solution is a least-squares fit of the Hill equation, $I = I_{\text{max}} C^n / (C^n + K_{1/2}^n)$, where I is current response, I_{max} is maximum current, C is odorant concentration, $K_{1/2}$ is the concentration required to elicit half the maximum response, and n is the Hill coefficient. The curve is fit with $I_{\text{max}} = 82$ pA, $K_{1/2} = 625$ μM , $n = 2.8$. The smooth curve in low- Ca^{2+} Ringer solution is fit with $I_{\text{max}} = 217$ pA, $K_{1/2} = 329$ μM , $n = 1$. The dashed line indicates that the foot of the dose-response relation is linear. (D) Responses of a different cell in low- Ca^{2+} Ringer solution to a 25-ms pulse of cineole at 100, 300, 500, 750, and 1000 μM , respectively. Each trace represents the average of five stimulus trials. (Inset) Linear dose-response relation. (E) Responses of a different cell in normal Ringer solution to a 200 μM cineole pulse of different durations (25, 35, 45, 55, 65, and 75 ms, respectively). Each trace represents the average of 10 stimulus trials. (Inset) Least-squares fit of the equation $I \propto C^n$ ($n = 2.8$). (F) Responses of a different cell in low- Ca^{2+} Ringer solution to a 100 μM cineole pulse of different durations (25, 35, 45, 55, 65, and 75 ms, respectively). Each trace represents the average of 6 to 10 stimulus trials. (Inset) A linear-regression fit has a time intercept of -2 ms. Results similar to those in Fig. 1, B, D, and F, were obtained upon “clamping” the internal Ca^{2+} concentration during the olfactory response by replacing external Na^+ in the low- Ca^{2+} solution with the permeant guanidinium ion to simultaneously stop Ca^{2+} influx through the CNG channel and Ca^{2+} efflux through the Na-Ca exchanger (11,24). (G and H) Strong dependence of $K_{1/2}$ of the dose-response relation on the duration of stimulation by odorant. Each panel represents responses from a different cell. Each point is the average of 2, 5 or 10 stimulus trials. (G) Normal Ringer solution. Relation between response amplitude and odorant concentration with stimulus durations of 25, 50, and 500 ms, respectively. The smooth curves are Hill-equation fits with I_{max} , $K_{1/2}$, and n of 75 pA, 1.5 μM , and 0.8 (500 ms); 61 pA, 99 μM , and 1.5 (50 ms); and 46 pA, 238 μM , and 2.3 (25 ms), respectively. Thus, by increasing the odorant duration from 25 to 500 ms, the $K_{1/2}$ decreases from 238 to 1 μM . (H) Low- Ca^{2+} Ringer solution. Dose-response relations from a different cell with 25- and 300-ms odorant duration, respectively. Smooth curves are Michaelis-equation (i.e., Hill equation with $n = 1$) fits, with I_{max} and $K_{1/2}$ of 121 pA and 24 μM (300 ms) and 45 pA and 128 μM (25 ms), respectively.

Fig. 2.

Quantal analysis of the olfactory response in 100 nM Ca^{2+} Ringer solution to a series of 190 identical weak pulses of cineole (50 ms, 50 μM). **(A)** Sample traces showing trial-to-trial fluctuations in the response amplitude. The red traces are scaled fits of a mathematical function (12) that describes the averaged response. **(B)** Amplitude histogram for 190 trials. Bin-width is 0.2 pA. **(Inset)** Ensemble mean and variance as a function of time. Downward spike in the mean response indicates junction current introduced to mark the timing and duration of odorant stimulation (12). At the response peak, the mean current was 2.9 pA and the variance was 2.6 pA^2 . These values give a unitary response of 0.9 pA and a mean quantal content (λ) of 3.2. The solid red curve is the Poisson distribution (12) with $\lambda = 3.2$, scaled by a unitary amplitude of 0.9 pA and blurred by Gaussian functions with $\sigma_0 = 0.27$ pA, $\sigma_1 = 0.33$ pA (12). The dashed profiles are Gaussians corresponding to failures and populations with quantal content of 1, 2, etc. **(C)** Results from 19 cells on the unitary amplitude in 100 nM Ca^{2+} , derived from σ^2/m . The values were all very similar and independent of m . Filled triangle and error bars: mean $\pm$ SD.

Fig. 3.

Variance analysis of the olfactory response in Ringer solution containing $20 \mu\text{M} \text{Ca}^{2+}$. (**A** to **C**) A series of 78 identical pulses of cineole (25 ms, $300 \mu\text{M}$) was delivered to an ORN. (**A**) Eight sample traces showing trial-to-trial fluctuations in the response amplitude. The red traces are scaled fits of a mathematical function that describes the averaged response. Downward spike in each trace indicates junction current introduced to mark the timing and duration of odorant stimulation (12). (**B**) (Left) Ensemble variance and mean as a function of time from 78 trials. The downward deflection in the “mean” trace was the junction current. The variance (σ^2) and (mean) 2 time courses overlap throughout. (**C**) The amplitude histogram was well described by the Poisson distribution calculated from the σ^2/m analysis (mean number of quanta = 4.0, quantal amplitude = 0.42 pA). (**D**) Variance/mean analysis of olfactory response from a different cell in four stimulus conditions, each with 20 identical cineole pulses. The value of σ^2/m is approximately constant at different m values. The Ringer solution contained guanidinium with a low concentration of Ca^{2+} . (**E**) Results of σ^2/m measurements, with cineole (27 cells), isoamylacetate (7 cells), or acetophenone (6 cells) as odorant. Black open squares show measurements in low- Ca^{2+} sodium Ringer solution. Red open circles show measurements

in low- Ca^{2+} guanidinium Ringer solution. Corresponding filled symbols show mean $\pm$ SD (black: 0.40 ± 0.06 pA, 18 cells; red: 0.17 ± 0.07 pA, 22 cells). The smaller unitary response in guanidinium/low- Ca^{2+} solution reflects a smaller inward current carried by guanidinium ion through CNG channels (25).

Fig. 4.

(A) Unitary responses for two odorants with different potencies on the same cell are very similar. (Top) Relation between response amplitude and odorant concentration for acetophenone and cineole odorants. Each point represents the average of four to eight stimulus trials. Although the duration of acetophenone stimulation was twice as long as that for cineole, the response with all receptors bound by acetophenone was a factor of 7 less than the response to cineole. (Bottom) Variance/mean analysis of the unitary response to the two odorants. The quantal responses to the two odorants were similar (0.48 pA for cineole and 0.56 pA for acetophenone). Thirty trials each of 100 μ M cineole at 25-ms duration and 2 mM acetophenone at 50-ms duration. The two stimuli were chosen to produce responses of comparable amplitudes. The slight difference in response kinetics for the two odorants was due to a change in cell condition during the experiment; this was not observed in other experiments. We chose this cell because of the large difference in efficacy between the two odorants. (B) Estimation of cineole dwell-time on the receptor. (Top) Relation between response amplitude and cineole concentration at two durations. Even when all receptors were bound (≥ 1 mM cineole), the response amplitude increased linearly with the odorant pulse duration. Each point represents the average of 3 to 20 stimulus trials. (Bottom) Complete data from the same experiment at a

saturating cineole concentration of 2 mM and applied for four different durations. **(Inset)**
 Linear increase of the response with odorant duration. The time intercept of the linear-
 regression fit is near zero.