Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 7864–7868, August 1995
Neurobiology

Olfactory transduction is intrinsically noisy

(cyclic AMP/olfaction/receptors/noise)

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Communicated by Denis A. Baylor, Stanford University School of Medicine, Stanford, CA, May 5, 1995 (received for review January 30, 1995)

ABSTRACT **The sources of noise that limit olfactory signal detection were investigated in dissociated rat olfactory receptor cells. Near-threshold odorant-evoked currents exhibited large random fluctuations. However, similar fluctuations were observed in the absence of applied odorants when currents were induced by elevating the intracellular cyclic AMP concentration. This suggests that the fluctuations reflect noise intrinsic to the transduction mechanism, rather than the quantal nature of an odorant stimulus. For many odorants, this intrinsic noise may preclude the reliable detection of single odorant molecules.**

Noise is a critical property of sensory transduction mechanisms because it limits the minimum stimulus that can be reliably detected. In vertebrate olfactory receptor cells, membrane current noise is quite small in the absence of odorants (see traces in refs. 1–3). This property might appear to indicate that olfactory transduction is a low-noise process, capable of detecting very weak stimuli. However, the observed baseline current noise does not accurately reflect the magnitude of biochemical transduction noise occurring in the absence of stimuli. This is because activation of a current by the intracellular messenger cyclic AMP is highly nonlinear (3), exhibiting an ≈4th (or higher) power dependence on cyclic AMP concentration. This nonlinearity approximates a threshold and therefore will attenuate baseline current noise caused by spontaneous fluctuations in the basal cyclic AMP concentration. The existence of this threshold was unexpected because it must decrease receptor sensitivity. Therefore, we proposed that the threshold serves to attenuate basal transduction noise (3).

We have now tested this hypothesis by measuring the membrane current noise evoked by near-threshold stimuli. Fluctuations in the odorant-evoked current could reflect basal transduction noise (intrinsic noise) but may also contain shot noise generated by the discrete activation of receptor proteins by single odorant molecules (quantal noise). We determined the relative magnitudes of intrinsic and quantal noise by comparing the noise evoked by cyclic AMP (in the absence of odorants) with the noise evoked by odorants. Olfactory transduction for many odorants is mediated by odorant binding to receptor proteins (4), which activate type III adenylyl cyclase (5–10) via an olfactory-specific G protein, G_{olf} (11). The resulting rise in cyclic AMP concentration generates an inward current by activating cyclic nucleotide-gated channels (12–16), with consequent activation of Ca^{2+}-dependent Cl^- channels (17, 18). According to this mechanism, if the fluctuations are quantal in origin, then they should appear in the current evoked by odorants but not in the current evoked by cyclic AMP. On the other hand, if the fluctuations reflect intrinsic noise, they should appear in the currents evoked both by odorants and by cyclic AMP. A preliminary report of these results was presented at the 1994 Society for Neuroscience Meeting (19).

MATERIALS AND METHODS

Odorant-evoked currents were recorded from dissociated rat olfactory receptor cells under whole-cell voltage clamp as described (3). Photolysis of caged cyclic AMP was performed as described (16). The odorants used in this study [menthone (85% (−)-menthone/15% isomenthone), 2-hexylpyridine, 2-isobutyl-3-methoxypyrazine, and isoamyl acetate] were chosen because cells responsive to them may be relatively abundant in the olfactory epithelia of air-breathing vertebrates (10).

Cells were stimulated by pressure-ejecting aqueous solutions of a single odorant or 3-isobutyl-1-methylxanthine (IBMX) from a micropipette, the tip of which was usually located at least 30 μm from the cell. The timing of the pressure pulses or steps is shown above the current traces in the figures. Ejection pressure and the cell–micropipette separation distance were adjusted to obtain responses of the desired magnitude. Stimulus concentrations given in the figure legends were those in the pipette, but concentrations at the cell should have been 1/10th to 1/1000th of those in the pipette, judging from previous studies using K^+-evoked currents to calibrate the odorant concentration at the cell (2). A more accurate calibration of the stimulus was not obtained because the conclusions of this study depend on the properties of current fluctuations at response threshold, not on the absolute odorant concentration.

The odorant concentrations in the stimulus micropipette are expressed as fractions of saturated aqueous solutions because measurements of aqueous solubility have not been reported for the odorants used. The maximum solubility of menthone, the odorant used for most experiments, was estimated to be 3 mM by inspection of menthone/saline mixtures for phase separation. Thus, the micropipette solution used—e.g., in Fig. 2*A*, trace 2—contained ≈3 μM menthone, which provided 3–300 nM at the cell surface, assuming a 10- to 1000-fold dilution of the micropipette solution at the cell surface (see above).

The number of odorant molecules that this stimulus provided to the cell is potentially important for determining the origin of discrete fluctuations, such as those in Fig. 2*A*, trace 2. For example, if the number of molecules were comparable to the number of discrete fluctuations, this would support interpreting these fluctuations as single molecular, or quantal, responses. However, if the number of molecules were much larger than the number of discrete fluctuations, this would argue against interpreting them as quantal responses. The number of molecules provided to the cell was estimated by assuming that odorant–receptor interaction is an efficient process and therefore is limited by diffusion. The steady-state diffusional flux to the surface of the odorant-sensitive cilia was calculated by approximating each cilium by a perfectly absorbing, highly elongated ellipsoid of revolution, with semi-axes *a*

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Abbreviation: IBMX, 3-isobutyl-1-methylxanthine.

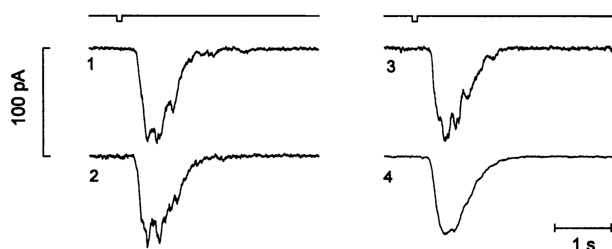

FIG. 1. Fluctuations in the odorant-induced current of a rat olfactory receptor cell evoked by 90-ms pulses of the odorant menthone. Traces 1–3 are individual responses to identical pulses; trace 4 is the mean current computed by averaging 13 such responses.

$\gg b$ (20). This calculation provides a lower bound for the rate of odorant–cell interaction for the following reasons: (*i*) it ignores multiple collisions between an odorant molecule and the cell surface, which are likely to occur (21); and (*ii*) the steady-state flux must be smaller than the flux that occurs immediately after an increase in odorant concentration. For the ellipsoidal geometry, the steady-state diffusional flux is given by $4\pi DaC/\ln(2a/b)$, where D is the odorant diffusion coefficient (assumed to be 5×10^{-6} $cm^2 \cdot s^{-1}$) and C is the odorant concentration, held constant at infinite distance (20). For a single rat cilium with diameter 0.1 μm (= $2b$) and length 30 μm (= $2a$), an odorant concentration of 3–300 nM (see above) provides a molecular flux of at least 3×10^4 to 3×10^6 s^{-1}. Therefore, for a cell with 10 cilia, the total molecular flux that can be detected is at least 3×10^5 to 3×10^7 s^{-1}. This rate is $>10^5$–10^7 times larger than the rate of discrete current fluctuations shown in Fig. 2*A*, trace 2, which argues against these events being quantal responses. More direct evidence regarding the origin of these fluctuations will be presented below.

All experiments were performed at 23°C except for those in Fig. 4 *A* and *B* for which the temperature was 30°C.

RESULTS

In ≈60% (15/24) of cells responding to the odorant methone, brief, suprathreshold stimuli evoked a transient inward current that displayed pronounced random fluctuations about the mean waveform (Fig. 1). During a response, the amplitude of the fluctuations increased with the mean current, reaching a peak value of 10 pA, rms, for the cell in Fig. 1, which was about six times larger than the baseline noise (1.6 pA, rms). The amplitude of the fluctuations varied between cells, the largest being ≈10 pA, rms. Similar fluctuations were observed in cells responsive to three other odorants: 2-isobutyl-3-methoxypyrazine (3/4 cells), 2-hexylpyridine (1 cell), and isoamyl acetate (1/2 cells).

Weaker stimuli of longer duration produced isolated random fluctuations in the baseline current that increased in frequency with increasing odorant concentration (Fig. 2*A*, traces 1–3). Expanded segments of the traces in Fig. 2*A* show that these isolated events did not exhibit a stereotypical amplitude or time course (Fig. 2*B*); a histogram of current amplitude exhibited a single broad peak, further indicating a lack of stereotypical events (data not shown). At higher odorant concentrations, the events merged into continuous noise (Fig. 2*A*, traces 4 and 5). The fluctuations decreased with further increases in odorant concentration (Fig. 2*D*). In the larger responses, the mean current decayed in the continued presence of the odorant due to adaptation (22, 23) (Fig. 2 *A*,

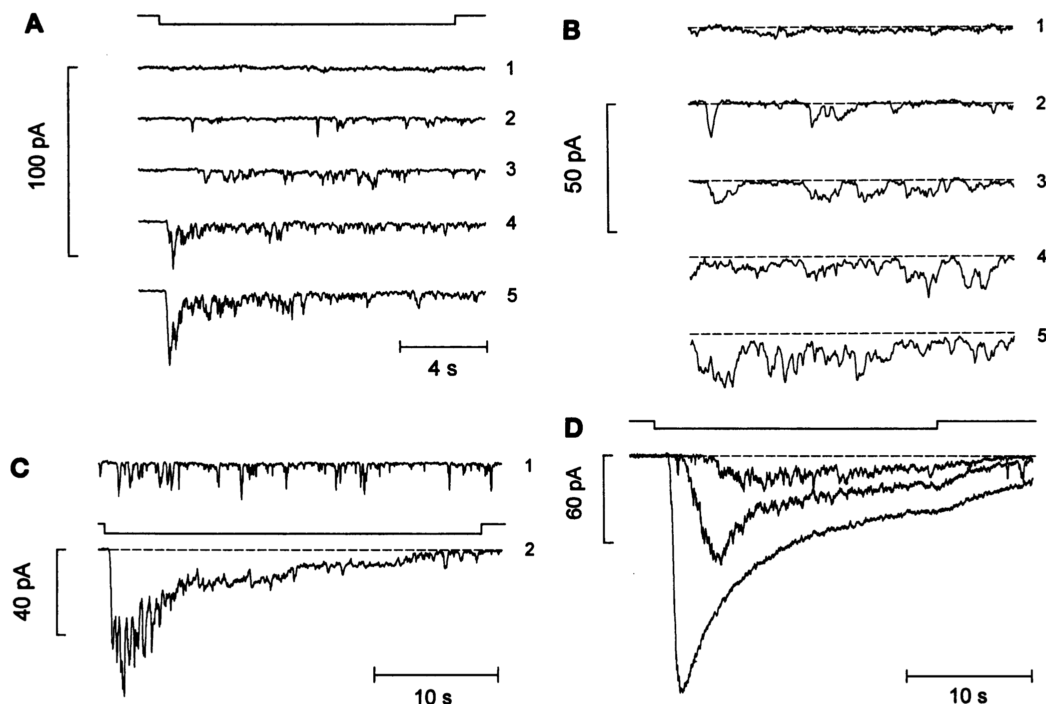

FIG. 2. Current fluctuations elicited by step odorant stimuli. (*A*) Responses of a cell to a series of steps of increasing menthone concentration varied by changing the distance between the stimulus micropipette and the cell (the stimulus ejection pressure was fixed at 15 psi; 1 psi = 6.89 kPa); the concentration is increasing from top to bottom; for trace 1 there was no stimulus. The menthone concentration in the stimulus micropipette was a 1000-fold dilution of a saturated solution. (*B*) Selected segments of each trace in *A*, of 3-s duration, expanded to show the current fluctuations. (*C*) Responses of a cell to menthone, showing the fluctuations at threshold (upper trace: 10^4-fold dilution of menthone, at 12 psi) and during adaptation to a larger response (lower trace: 1000-fold dilution of menthone, at 17 psi). The stimulus trace applies only to the lower data trace. For the upper data trace, the stimulus was on during the entire recording period. (*D*) Decrease in noise during larger responses recorded from a third cell during steps of increasing odorant concentration varied by moving the stimulus micropipette, as in *A* (100-fold dilution of 2-isobutyl-3-methoxypyrazine, at 3 psi). All traces were low-pass filtered digitally (3 dB cutoff at 31 Hz). The data in *A*, *C*, and *D* were derived from different cells.

C, and *D*). The fluctuations also decayed during adaptation and in some cells became smaller than the fluctuations at threshold (Fig. 2*C*, compare traces 1 and 2).

The origin of these fluctuations was investigated by comparing the fluctuations evoked by odorants with the fluctuations evoked by cyclic AMP in the absence of odorants (see the Introduction). Cyclic AMP-evoked currents were elicited either by exposure to the phosphodiesterase inhibitor IBMX (3), or by photolysis of caged cyclic AMP (3, 16). Fig. 3 *A* and *B* shows responses of similar amplitude evoked by stepped application of the odorant menthone or IBMX. Conspicuous fluctuations are present in both responses. Similar fluctuations were observed in currents evoked by cyclic AMP released by photolysis of caged cyclic AMP (Fig. 3*C*). Both odorant and IBMX responses to prolonged stimuli decayed due to adaptation, although the rates and magnitudes of decay varied between cells. The current induced by prolonged photolysis of caged cyclic AMP decayed even more rapidly. These differences in adaptation kinetics may reflect the multiplicity of putative adaptation mechanisms demonstrated in olfactory receptor cells (24–30).

The fluctuations evoked by different stimuli were compared by calculating their power spectra from data such as those in Fig. 3. These spectra cannot be used to estimate physical parameters that characterize the noise source because the responses were nonstationary and subject to nonlinear amplification by Ca^{2+}-dependent Cl^- channels (3). However, they do provide a quantitative basis for comparing fluctuations caused by different stimuli, provided the amplitudes and time courses of the mean currents are similar. Furthermore, nonstationarity could not have had a large effect on the shapes of the power spectra because similar power spectra were obtained in the few cases where stationary fluctuations were observed (see legend to Fig. 4*B*). Fig. 4 *A* and *B* shows the power spectra of fluctuations in odorant- or IBMX-induced currents, both recorded from the same cell. The spectra were almost identical in their shape; both declined more steeply than a Lorentzian function, with an approximate $f^{-2.5}$ frequency dependence, and corner frequencies between 1 and 3 Hz. The amplitudes of the spectra were identical above ≈5 Hz but differed at lower frequencies, where more power was present in the IBMX-induced current. This may be due in part to the slightly larger mean amplitude of the IBMX response, which would magnify the fluctuations through the nonlinear cyclic AMP dependence of the current. Could the fluctuations in the IBMX response simply be due to IBMX-induced fluctuations in phosphodiesterase activity or to IBMX acting as an odorant? Neither alternative is likely because the photolysis response also exhibited fluctuations with a similar power spectrum (Fig. 4*D*). Independent evidence that IBMX acted directly on the phosphodiesterase rather than on receptor proteins comes from the following observations: (*i*) the latency of IBMX responses was consistently shorter than the latency of odorant responses (3) and (*ii*) IBMX responses decreased more rapidly than odorant responses when the stimulus was truncated by a brief pulse of Ringer's solution (data not shown). IBMX might be expected to slow the noise kinetics because IBMX prolongs transient odorant responses (31). We attribute the absence of this effect to a low concentration of IBMX enveloping the cell, as indicated by the small amplitude of the IBMX-evoked current compared with previous recordings in which the tip of the stimulus micropipette was closer to the cell and higher ejection pressures were used (3). It might also be supposed that the fluctuations were caused by odorous impurities in our solutions. However, this predicts that the fluctuations evoked by odorants would be larger than the fluctuations evoked by either cyclic AMP or IBMX, which was not observed.

To explore further the dependence of the current fluctuations on the nature of the stimulus, we compared the responses of a cell to two structurally dissimilar odorants; menthone and 2-hexylpyridine. Different odorants might generate different noise spectra owing to their different affinities for the receptor proteins (32). However, as shown in Fig. 4*C*, the power spectra of the fluctuations induced by these odorants were indistinguishable.

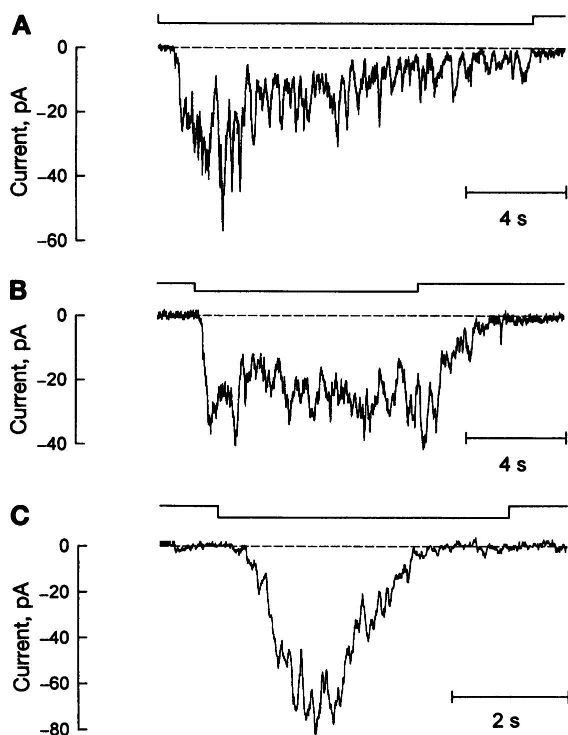

FIG. 3. Comparison of fluctuations observed during responses of different cells to stepped application of menthone (100-fold dilution; 18 psi) (*A*), IBMX (2.5 mM; 12 psi) (*B*), and cyclic AMP (*C*), caused by photolysis of 100 μM caged cyclic AMP [light step intensity attenuated by 2.3 log units (15)]. In *C*, the stimulus trace indicates the time during which the cell was exposed to UV light. The data shown in *A*, *B*, and *C* was derived from different cells.

DISCUSSION

We have shown that the currents evoked by odorants and by cyclic AMP exhibit similar fluctuations in rat olfactory receptor cells. Therefore, these fluctuations appear to reflect noise intrinsic to the olfactory transduction mechanism rather than quantal activation of transduction by single odorant molecules (see the Introduction).

What is the source of this intrinsic noise? For many odorants, the transduction current is mediated by an increase in cyclic AMP concentration which activates cyclic nucleotide-gated channels (12–16) with consequent activation of Ca^{2+}-dependent Cl^- channels (17, 18). Therefore, intrinsic transduction noise could reflect fluctuations in second messenger concentration, ion channel noise, or both. Ion channel noise, however, is unlikely to account for our data because this would predict a fixed relationship between the mean and the variance of the current, which was not observed—e.g., Fig. 2*C*, compare trace 1 with the end of trace 2. Therefore, we propose that the intrinsic noise is due to spontaneous fluctuations in second messenger concentration, probably in the basal concentration of cyclic AMP. Spontaneous fluctuations in cyclic AMP concentration could occur because of the high basal adenylyl cyclase activity in the absence of odorants, demonstrated both in biochemical measurements on purified cilia (7) and in intact cells, as indicated by the ability of IBMX to evoke currents as large as those evoked by odorants (3). Another reason to suspect spontaneous fluctuations in the basal cyclic AMP

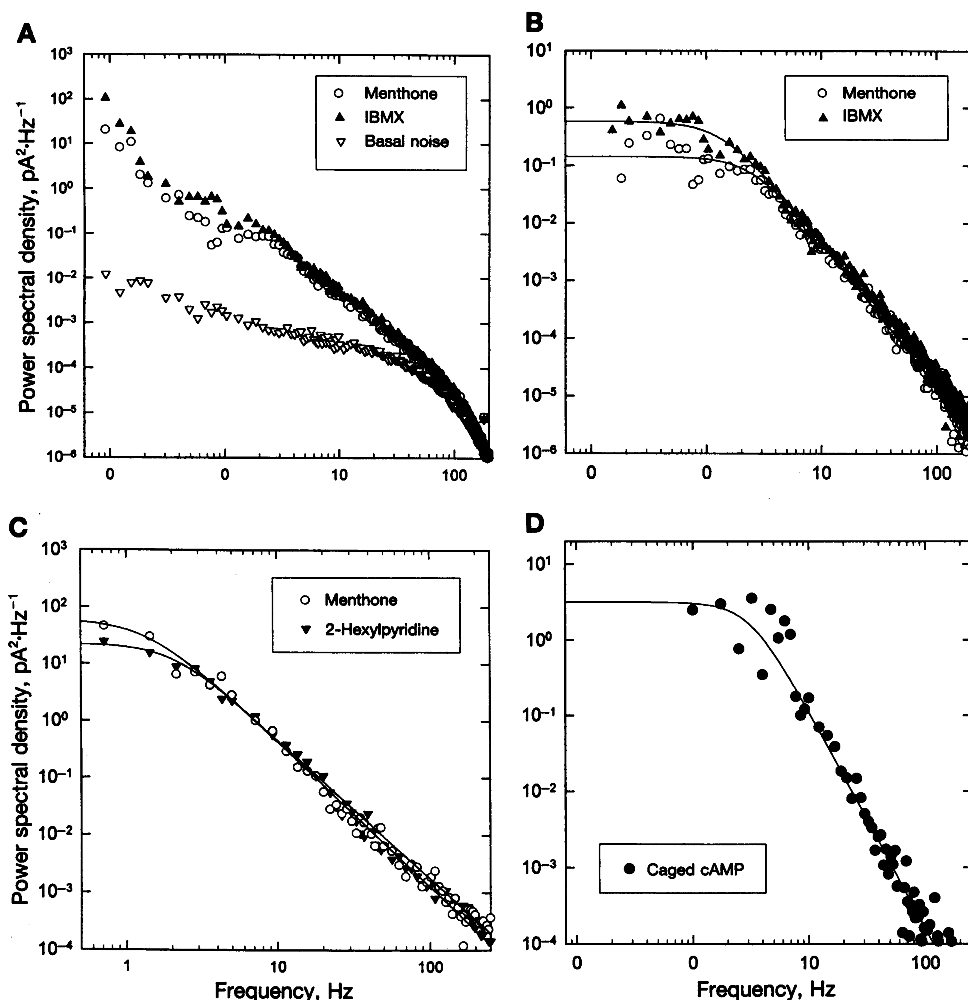

FIG. 4. Power spectra of fluctuations in responses to stepped application of odorants, menthone, or IBMX. (*A*) Raw power spectra of traces, including responses to menthone or IBMX, and of the baseline current in the absence of stimuli. The power spectra for menthone and IBMX each represent an average of three spectra computed from three responses to step stimuli recorded from the same cell (which was different from the cells shown in Fig. 3). The power spectrum of the baseline noise was computed from six current traces acquired from this cell in the absence of stimuli. (*B*) Power spectra of the fluctuations in the responses to menthone or IBMX for the cell shown in *A*. The fluctuations were isolated by subtracting an estimate of the mean current from individual traces. The power spectrum of the baseline noise was then subtracted from the power spectrum of the fluctuations, and the resulting power spectrum was divided by the square of the transfer function of the 8-pole Bessel filter used for antialiasing. The mean current was estimated by fitting an 8- to 12-order polynomial to the traces, which was about the minimum order required to fit traces exhibiting little noise. It was not possible to estimate the mean current by collecting an ensemble average because of the short durations of most recordings (10–20 min). In *A* and *B*, responses to 22-s stimuli were sampled every 2 ms, with the 3 dB cutoff frequency of the filter set to 125 Hz. Each point from 0.2–1 Hz is the average of 3 raw frequency points, from 1–10 Hz, the average of 9 points, and above 10 Hz, the average of 27 points. The continuous curves fitted to the data were $A/[1+(f/f_0)^n]$, where A = 0.14 pA²·s, f_0 = 2.54 Hz, and n = 2.50 for menthone; and A = 0.58 pA²·s, f_0 = 1.34 Hz, and n = 2.37 for IBMX. The parameters for a stationary response from one cell to menthone were the following: A = 0.94 pA²·s, f_0 = 0.99 Hz, and n = 1.93. (*C*) Power spectra of the fluctuations in the responses to the odorants menthone and 2-hexylpyridine, recorded from the cell shown in Fig. 1. A series of consecutive responses to transient odorant stimuli (90-ms pulses for menthone, 120-ms pulses for 2-hexylpyridine) were recorded (13 responses for menthone, 14 for 2-hexylpyridine), and the mean responses were subtracted to yield traces for calculation of averaged power spectra. Averaged baseline noise spectra were computed from traces recorded between each stimulus and subtracted from the power spectra of the fluctuations. The duration of the responses was 1.6 s for both stimuli. Responses were sampled every 1 ms, with the 3 dB cutoff frequency of the filter set to 250 Hz. Points below 5 Hz are raw frequency points, those from 5–50 Hz, the average of three points, and those above 50 Hz, the average of nine points. Continuous curves were fit to the data as in *A*, with the following parameters: A = 59.2 pA²·s, f_0 = 1.34 Hz, and n = 2.41 for menthone; and A = 22.6 pA²·s, f_0 = 2.13 Hz, and n = 2.54 for 2-hexylpyridine. (*D*) Power spectrum of the photolysis response from the cell shown in Fig. 3*C*. Curve fit parameters were as follows: A = 3.15 pA²·s, f_0 = 3.14 Hz, and n = 2.86.

concentration is that this might explain the pronounced threshold in the current evoked by cyclic AMP (3). A threshold was unexpected because it decreases receptor-cell sensitivity. However, it would have the benefit of improving the signal-to-noise ratio in second-order neurons. This is because, as argued by Baylor *et al.* (33) for the retina, a threshold would prevent the summation of transduction noise in second-order neurons, thereby improving the signal-to-noise ratio in these neurons when only a fraction of the receptor cells are activated. This argument is particularly relevant to olfaction because of the high convergence of olfactory receptor axons in the olfactory bulb (34). Thus, we propose that the threshold for current generation by cyclic AMP (3) serves to prevent spontaneous fluctuations in the basal cyclic AMP concentration from generating a current.

According to our hypothesis, discrete events, such as those shown in Fig. 2 *A–C*, merely represent the suprathreshold peaks in a continuum of spontaneous fluctuations rather than intermittent activation of the transduction cascade. This intrinsic noise is attenuated in the absence of stimuli because the

fluctuations normally fall below the threshold for current generation. However, any stimulus that elevates cyclic AMP concentration, be it an odorant or a pharmacologic agent, will reveal these biochemical fluctuations as a current.

What could cause spontaneous fluctuations in the basal cyclic AMP concentration? One possible explanation is spontaneous (thermal) activation of the olfactory receptor proteins, G_{olf}, or both. Spontaneous activation of ligand-activated receptors is a necessary consequence of the equilibrium between active and inactive conformations (35), and several examples of this phenomenon have already been demonstrated (36-38). Spontaneous activation of olfactory receptor proteins or G_{olf} is, in fact, suggested by the high basal adenylyl cyclase activity in the native membrane (7), compared with the very low basal activity observed when the cyclase is expressed in a human kidney cell line (8). An additional cause of spontaneous fluctuations might be Ca^{2+}-feedback control of cyclic AMP metabolism: Ca^{2+} influx via cyclic nucleotide-gated channels (22, 39) can reduce cyclic AMP concentration both by inhibition of adenylyl cyclase (6, 7) and by stimulation of phosphodiesterase (24). A similar feedback pathway is responsible for low-frequency noise in the dark current of vertebrate rods (40).

Whatever the source of the intrinsic noise, the fact that it dominates the fluctuations observed in near-threshold odorant responses (as shown by the similarity of the power spectra in Fig. 4 *A* and *B*) prevents discrimination between quantal and intrinsic noise, and therefore should preclude the detection of single odorant molecules. This does not, of course, rule out the possibility that single odorant molecules may be detected in cells exhibiting higher sensitivity or lower intrinsic noise than those studied thus far. However, given that most vertebrate receptor cells have thresholds in the range of 10^{-6} M to 10^{-9} M (ref. 41, but compare ref. 42) and the large number of odorant molecules that such stimuli provide to a cell (see *Materials and Methods*), we suggest that quantal detection is not a common property of vertebrate olfactory receptor cells.

Note Added in Proof. After submission of this manuscript, Menini *et al.* (43) reported current fluctuations in salamander receptor cells that appear similar to those reported here. In contrast to our study, they concluded that the fluctuations reflect quantal activation of transduction by single odorant molecules. However, their observations can, in our opinion, be explained equally well by intrinsic transduction noise combined with the threshold for current generation. For example, "quantal-like" current bumps could be generated by fluctuations in cyclic AMP concentration that only occasionally exceed the threshold. "Failures" in responses to pulsed stimuli could result when an odorant stimulus coincided with a downward fluctuation in cyclic AMP concentration. Their quantal interpretation is undermined further by the high odorant concentrations used and the nonlinear summation of quantal-like fluctuations, which would be expected to sum linearly (44). Thus, we suggest that their observations reflect intrinsic noise rather than quantal responses.

We thank Drs. D. A. Baylor, T. D. Lamb, T. Kurahashi, L. M. Masukawa, E. N. Pugh, Jr., and T. Sato for valuable comments on the manuscript and E. Meisami for helpful discussions. Supported by National Institutes of Health Grant DC00505.

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