Functional Asymmetries in Cockroach ON and OFF Olfactory Receptor Neurons

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Burgstaller M, Tichy H. Functional asymmetries in cockroach ON and OFF olfactory receptor neurons. J Neurophysiol 105: 834–845, 2011. First published December 15, 2010; doi:10.1152/jn.00785.2010. The ON and OFF olfactory receptor neurons (ORNs) on the antenna of the American cockroach respond to the same changes in the concentration of the odor of lemon oil, but in the opposite direction. The same jump in concentration raises impulse frequency in the ON and lowers it in the OFF ORN and, conversely, the same concentration drop raises impulse frequency in the OFF and lowers it in the ON ORN. When the new concentration level is maintained, it becomes a background concentration and affects the responses of the ON and OFF ORNs to superimposed changes. Raising the background concentration decreases both the ON-ORN’s response to concentration jumps and the OFF-ORN’s response to concentration drops. In addition, the slopes of the functions approximating the relationship of impulse frequency to concentration changes become flatter for both types of ORNs as the background concentration rises. The progressively compressed scaling optimizes the detection of concentration changes in the low concentration range. The loss of information caused by the lower differential sensitivity in the high concentration range is partially compensated by the higher discharge rates of the OFF ORNs. The functional asymmetry of the ON and OFF ORNs, which reflects nonlinearity in the detection of changes in the concentration of the lemon oil odor, improves information transfer for decrements in the high concentration range.

INTRODUCTION

Olfactory receptor neurons (ORNs) in insect antennal sensilla exhibit characteristic levels of spontaneous activity and odors can cause either an increase (excite) or a decrease (inhibit) of their action potential firing frequency (Ache and Young 2005; Hallem et al. 2004; Madrid et al. 2001; Nakagawa and Vosshall 2009). A spontaneous discharge is thus the “null” condition and any change in the flux of the action potential—whether positive or negative—can signify some relevant parameter of the odor stimulus. The lower the impulse frequency, the longer it takes to convey information. If the odor concentration shifts so rapidly that its direction of change can vary during the period needed for an inhibited ORN to transmit the extent or speed of the concentration change, this ORN will not be able to keep up. The response will be interrupted and therefore less accurate.

In many sensory systems there are complementary and reciprocal sets of neurons that form symmetrically opposing pairs. Accordingly, in vision, blackness turns one set of retinal ganglion cells on and stops another set; illumination excites those retinal ganglion cells that were inhibited by darkness, but inhibits those that were excited by a black stimulus (Schiller 1992; Schiller et al. 1986). The ON and OFF dichotomy does not exist in the photoreceptor cells themselves, but originates at the bipolar cell level of the retina.

In the olfactory system of the cockroach, ON and OFF responses were demonstrated directly at the level of the antennal ORNs. A specific hairlike sensillum houses two types of ORNs that are activated by the same change in the concentration of the odor of lemon oil, but in the opposite direction (Hinterwirth et al. 2004; Tichy et al. 2005). The rate of discharge of one type, the ON ORN, is increased by raising odor concentration and decreased by reducing it. The discharge rate of the second type, the OFF ORN, is increased by reducing odor concentration and decreased by raising it. Since during both increments and decrements in odor concentration the frequency of one of the two ORNs is always high, information can be supplied on and during both directions of change by one or the other ORN.

The separation of olfactory information into parallel ON and OFF ORNs enhances the contrast between two neighboring regions of different odor concentration by signaling “higher concentration than background” and “lower concentration than background.” This arrangement suggests that it is as important for the foraging cockroach to detect jumps in the concentration of the odor of lemon oil within environmental volatiles that contain a low concentration of the lemon oil odor as it is to notice concentration drops of the same odor against a high background lemon oil odor concentration. There are no quantitative data regarding the spatial and temporal distribution of odors emitted by ripe citrus fruits. Most likely they continuously give off volatiles at high concentrations. In kitchens or storerooms, where odors are constantly present in the air, a sharp increase in fruit odor concentration will be perceived as an odor stimulus. When that concentration is maintained for a time, it will become a background concentration against which a change must be detected and quantified. Continuous exposure to background values can reduce or even eliminate the responsiveness to superimposed changes of the same odor stimulus, acting mainly through the process of self-adaptation (Dolzer et al. 2003; Schröder and Hilker 2008).

The present study examines the effect of background concentrations of lemon oil on the response of the ON and OFF ORNs to jumps and drops in that odor. We determined the resolving power of ON and OFF ORNs, that is, the precision with which an ORN can discriminate concentration changes. We analyzed the influence of the background concentration on the resolving power for such concentration changes. We further determined that both ORNs discharge continuously during the background odor. To clarify whether the contin-
uous discharge is spontaneous or even a tonic response to the background, we described for both types of ORNs the relationship between the discharge rate and the background concentration and we determined how accurately the continuous discharge permits discrimination of background levels. We show that the ON and OFF ORNs are not a symmetric system with equal and opposite responses, revealing nonlinearity in the detection of the lemon oil odor.

METHODS

Experimental animal and odor stimulus

The nocturnal cockroach Periplaneta americana has a highly developed olfactory sense. Combined structural and physiological investigations have revealed a fairly complete list of olfactory sensilla, their innervation pattern, and their distribution on the antenna (Altner et al. 1977, 1983; Fujimura et al. 1991; Schaller 1978; Toh 1977). Lemon is an effective stimulus not only for ORNs (Sass 1978) but also for antennal lobe neurons (Boeckh 1974; Selzer 1981, 1984; Zeiner and Tichy 2000). Since the quality of odor compounds in natural fruits can differ greatly depending on the region of origin, maturity, and storage, we used synthetic lemon oil (Roth, D ~ 0.85, Art. 5213.1; Hinterwirth et al. 2004; Tichy et al. 2005; Zeiner and Tichy 2000) as a standardized fruit odor stimulus.

Preparation

A male adult American cockroach was anesthetized with CO₂ before it was strapped to a Plexiglas holder with Parafilm. For the extracellular recordings from individual sensilla, one antenna was kept in a forward position by cementing it onto a ledge that extended from the holder, using small strips of gaffer tape and dental cement (Harvard Cement; Harvard Dental-Gesellschaft, Berlin). The thus immobilized but live cockroach was then placed in the experimental setup, so that the odor-delivery nozzle ended about 10 mm from the recording site on the antenna.

Olfactometer

Odor stimulation was provided by using a dilution flow olfactometer (Hinterwirth et al. 2004; Prah et al. 1995; Tichy et al. 2005). Compressed air was cleaned and divided into two streams. The first air stream was modulated by mixing the odor-saturated and the clean air streams in a ratio determined by the proportional valves by means of the output sequencer function of the data acquisition software (Spike2, v. 3.18; Cambridge Electronic Design [CED], Cambridge, UK), using a self-written sequencer script. The flow rate of the mixed air stream was held constant by shifting the phase of the control voltages (A-D converter, 1401plus; CED) of the proportional valves by 180°. A feedback linearization, which integrated the voltages used to control the proportional valve with those received from the flow meters, counteracted any deviations of the flow rate set by the output sequencer. The mixed air stream emerged at 1.5 ms⁻¹ from a 7-mm-diameter nozzle. Air stream velocity was measured by a hot wire anemometer. The recording site was situated 10 mm from the outlet of the tube. The air around the antenna was continually removed by a suction tube adjusted to a suction speed of 2 ms⁻¹.

Stimulus concentration was calculated using the flow rate ratio of odor-saturated air to clean air and indicated throughout by the percentage of the saturated air in the stimulus air stream leading to the cockroach: “0% saturated air” means clean air only and indicates that the air stream directed onto the cockroach does not contain the odor stimulus and “100% saturated air” means pure odorized air and indicates that the stimulus air is not mixed with clean air. By controlling the ratio of the flow rates of clean air and odor-saturated air with electronic flow meters, the olfactometer generated and delivered the same set of steady concentrations throughout the study. Concentration was termed “steady” when measurable concentration changes as indicated by the flow rate did not occur in the course of 60 s. Sometimes very low amplitude, low-frequency fluctuations involving 2% in 400 s (0.005%/s) did appear, however. No systematic attempt was made to determine whether such low rates of concentration change affect the discharge rate of the ON and OFF ORNs; if they do, the effects were not obvious. The ON and OFF ORNs definitely react to rates of concentration change in the order of 10%/s (Tichy et al. 2005), but this is 2 × 10⁻³ times faster than 0.005%/s. For rapid concentration changes, the difference between the background concentration and the stimulus concentration was used to indicate stimulus magnitude. A positive value (+Δconc) reflects the upward direction of the concentration change, a negative value the downward direction (−Δconc).

FIG. 1. Diagram illustrating the dilution flow olfactometer. Odor concentration is determined by bubbling air through the tank containing the liquid odorant (lemon oil) and mixing with clean air in proportions regulated by electrical valves. A, amplifier; CED (Cambridge Electronic Design) data acquisition interface; electrical valve 1/2, electrical proportional valves; FM 1/2, flow meters; I, indifferent electrode; R, recording or different electrode; FB-controller 1/2, feedback controller of proportional valves.
Recordings

Electrodes were electrophytically sharpened tungsten wires. The reference electrode was placed in the tip of the antenna; the recording electrode was inserted into the base of the sensillum. All recordings were taken from swC sensilla, which are single-walled basiconic sensilla (Altner et al. 1983; Hinterwirth et al. 2004; Schaller 1978; Tichy et al. 2005). Impulses were amplified and filtered (0.1–3 kHz), passed through a 1401plus A-D converter (CED), and fed into a PC. The digitized action potentials and the voltage output of the electronic flow meters were displayed on-line on a monitor, stored on a hard disk, and analyzed off-line using Spike2 software. Spike waveform parameters were extracted and sampled to form templates. Detected spikes were offered to the template-matching system to create or modify the templates. Each spike was compared against the templates and each time a template was confirmed it was added to the template by overwriting (Fig. 3C). Adding a spike to a template may change the shape and width of the template outlines. Thus the template boundaries displayed homogeneity of classification and informed about temporal peculiarities of the spike trains, such as a gradual change from one class to the other, resulting in erroneous counts in each class.

Response evaluation

Often it is unclear what neuronal code is used for a particular stimulus parameter, but the code for stimulus intensity is, in all likelihood, the least doubtful one. It is generally thought to be a “rate” or “frequency” code, the intensity being represented in the mean firing rate of the receptor neurons involved over time. To express the opposite responses of the ON and OFF ORNs on the same timescale, we determined impulse frequency (impulses/s) by impulse counts for fixed periods of 1 s. A previous study has shown that the ON and OFF responses to a given jump or drop in odor concentration, respectively, tended to rise numerically as progressively shorter periods of the spike trains are used to determine impulse frequency by emphasizing peak frequency values (Tichy et al. 2005). Short periods, however, decrease averaging time and increase impulse interval scatter. It became apparent that the higher discharge rates of the peak frequency values are cancelled out by a higher degree of scatter. The 1-s period was chosen as a representative parameter for response evaluation because the number of action potentials that they include appeared large enough to compensate for the effect of interval-to-interval scatter. Moreover, the chief concern here was not to compare the stimulating effect of the two stimuli be? The two stimuli can be a pair of constant-concentration values or a pair of concentration changes. A full mathematical development of the concepts underlying the resolving power (Δx) was presented by Loftus and Corbibre-Tichané (1981). The equation is

\[ \Delta x = \frac{\sqrt{2\pi}}{|b|} \Phi^{-1}(\gamma) \]

in which \( |b| \) is the mean absolute slope of the stimulus–response functions. Because the slope of a parabola varies continuously along the curve and the parabolas approximating these functions were not the same for all ORNs, \( |b| \) was obtained by taking the mean of the individual slopes (i.e., first differential) corresponding to the stimulus actually presented. \( \sigma^2 \) is the variance of the individual deviations of points about their respective regressions, \( \gamma \) is the required probability (90%), and \( \Phi^{-1}(\cdot) \) is the inverse of the distribution function of a standardized, normally distributed, random variable. \( \Phi^{-1}(0.90) = 1.28 \) (see tables in Diem and Lentner 1968). In the case of a linear regression, \( \sigma^2 \) is estimated by

\[ \sigma^2 = \frac{\sum e^2}{n - 2I} \]

and for a parabola by

\[ \sigma^2 = \frac{\sum e^2}{n - 3I} \]

where \( e \) is the deviation of each individual point from its curve, \( I \) is the number of curves, and \( n \) is the number of measurements. \( \sigma \) is reduced by the number of degrees of freedom, which is \( 2I \) because two estimates are necessary to determine each straight line \( (a + bx + c) \). Since the resolving power is calculated from parabolas, \( n \) is reduced by \( 3I \), corresponding to the three estimators for each parabola \( (a, b, \text{and } c) = a + bx + cx^2 \).

This method can be applied if the following conditions are met: 1) the deviations of the individual points from their curves must be
normally distributed about a mean of zero and 2) the absolute deviations (sign ignored) must not depend on the slope of the curves. The absolute deviations of single points from their regressions did not depend on the slopes of the regressions. However, their distribution was not normal (\(\chi^2\) test). Although bell-shaped, the flanks of the distribution curve were too steep; the points tended to be located too centrally. This type of distribution will, if anything, underestimate the resolving power. The normal distribution model was accepted for the lack of a better one.

The close proximity of the ON and OFF ORNs in the same sensillum allowed picking up the action potentials of both simultaneously with the same extracellular electrode. Clear differences in impulse amplitude permitted distinction of their activity without ambiguity, as exemplified in Fig. 3, A–C, a typical single-sensillum recording. The experiment involved jumps and drops in the concentration of the lemon oil odor (\(+\Delta_{\text{conc}}\) and \(-\Delta_{\text{conc}}\), respectively) and three constant background concentrations (back-conc). A test sequence always began with 0% back-conc, presented for 3 min. This was followed by a jump to 50% back-conc, also held for 3 min, and a second jump to 100% back-conc for 3 min. Then the sequence was reversed and odor concentration dropped from 100% to 50% and finally to 0% back-conc, each maintained

**RESULTS**

*Response profiles*

The absolute deviations of single points from their regressions did not depend on the slopes of the curves. Connecting the SDs above and below the means yields a band that contains 68% of the points, a percentage verified by actual count. In Fig. 2B, the band is shaded and indicates much less scatter than that in Fig. 2A. It reflects the preponderance of small deviations (Fig. 2B), which indicates an ability to distinguish small stimulus intensities with high probability. The error introduced by too large a value of variance (Fig. 2A) will result in a too conservative estimate of the resolving power.

**FIG. 3.** A–F: simultaneously recorded responses of a pair of ON and OFF ORNs from a single sensillum to a standard sequence of 3 constant odor concentrations (0, 50, and 100%), each maintained for 80 s. The change from one concentration level to the next was instantaneous. A: activity during the final 5 s of a 3-min presentation of clean air and after a jump to the 50% concentration level. Top: time course of the concentration of the lemon oil odor as monitored by an electronic flow meter; middle: responses of the ON and OFF ORNs represented in raster plots; bottom: simultaneously recorded impulse discharges of the ON and OFF ORNs. B: activity during the final 5 s of a 3-min presentation of a 50% constant odor concentration and after a drop to clean air. Top: time course of the odor concentration; middle: responses of the ON and OFF ORNs represented in raster plots; bottom: impulse discharges of the ON and OFF ORNs. C: template windows showing the template boundaries of the spike waveforms from the ON and OFF ORNs in the recordings in A and B. D: time course of the odor concentration as monitored by an electronic flow meter; E and F: impulse rates (bin width, 1 s) of the ON and the OFF ORNs, respectively. Stars, responses to 50% concentration jumps; triangle, responses to 50% concentration drops; F, impulse frequency; bin width, 1 s.

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again for 3 min. Figure 3D shows the order and time course of stimulus presentation.

As illustrated in Fig. 3E, the ON ORN responded to each 50% jump (+\(\Delta\)conc) with a rapid increase in discharge rate, followed by a slow decline to a constant activity level. The effect of the 50% +\(\Delta\)conc was most obvious at the 0% rather than the 50% back-conc (Fig. 3E, stars). Under the same conditions the OFF ORN fell silent for a short period; then its discharge resumed and gradually rose until a stable level was reached and maintained (Fig. 3F). On reversing the direction of concentration change, i.e., proceeding from the 100% to the 50% back-conc and then back to 0%, the OFF ORN produced a rapid increase in the discharge rate, followed by a slow decline to a constant value. The response to the 50% drop (−\(\Delta\)conc) was most pronounced at the 50% rather than the 100% back-conc (Fig. 3F, triangles). The ON ORN ceased discharging, followed by a slow return to a stable state discharge rate.

Responses of the ON ORN to concentration jumps

The ON and OFF ORNs examined as in Fig. 3 showed more than just response antagonism. The activity of an ORN to the same 50% jump in odor concentration (+\(\Delta\)conc) differed when initiated from different background values (back-conc). To determine the effect of the back-conc on the ON-ORN’s response to +\(\Delta\)conc required several series of +\(\Delta\)conc from different back-conc values. Since the effects are comparable only for equal values of +\(\Delta\)conc at each back-conc, the obvious procedure would be to record responses at constant values of +\(\Delta\)conc for every back-conc. However, the range of +\(\Delta\)conc that could be tested becomes progressively smaller when the back-conc is rising. Thus spacing of +\(\Delta\)conc from the different back-conc was not constant but varied between 13 and 20%. This separation was large enough to reveal a tendency in the course of +\(\Delta\)conc, yet small enough to render very low the probability of a significant bump or dip in the general course of the functions.

Figure 4A shows the results of one such experiment. Five responses to +\(\Delta\)conc were investigated from each of three back-conc values in the 0 to 40% range (that is at 0, 20, and 40% back-conc) and three responses from the 60% back-conc. The first stimulus of a series was tested only after each back-conc had been presented for ≥3 min. Then the ON ORN was exposed to 3-s periods of higher concentrations with intervals of 1 min at the back-conc. When such a series had been completed, the ORN was exposed to a new back-conc and the next series was begun. Impulse frequency (\(F\)) rose monotonically with the size of +\(\Delta\)conc, but more rapidly the lower the back-conc. A linear regression approximates the relationship of \(F\) to +\(\Delta\)conc quite well, but parabolic regressions even better. The slope of the parabolas flattens as +\(\Delta\)conc becomes larger. Furthermore, the effect of +\(\Delta\)conc on \(F\) decreases with increasing back-conc. As the equal-frequency line in Fig. 4A illustrates, it takes a 40% +\(\Delta\)conc to elicit 10 impulses/s at 40% back-conc, but only a 13% +\(\Delta\)conc at 0% back-conc.

Since the slope along a parabola (and thus the differential sensitivity) varies continuously over the range of +\(\Delta\)conc, no single slope value could be assigned to the entire segment of the parabola approximating a characteristic curve. Rather, slope values were provided by the first derivative of the parabola at each +\(\Delta\)conc used as stimulus. Thus each response has its own corresponding slope. For each slope, the mean value at the five points along the parabolic regression was computed and the mean and SD of the pooled responses plotted as a function of both parameters (Fig. 4B). Then, for each back-conc, the relationship between mean F values and +\(\Delta\)conc was approximated by a single parabolic regression and the mean slope (Fig. 4B, solid line) and its SD for the five points were determined (Table 1Aa). Connecting the SDs above and below the means yields a band that contains 68% of the points in each series, a percentage verified by actual count. Such a band suggests the degree of scatter of individual F values about the mean response curve. At back-conc levels of 0 and 40%, for example, the mean slope values of the five points along the parabolas are 0.22 ± 0.06 and 0.11 ± 0.01 (impulses/s)/\(\Delta\)%, respectively (Table 1Aa). The CD values

\[A\] single ON ORN
\[B\] 13 ON ORNs

\[\text{FIG. 4.} \ A \text{ and} B: \text{ responses of ON ORNs to concentration jumps (+\(\Delta\)conc) from 4 different background concentrations (back-conc). The slope of the parabolic regressions approximating the course of each set of F values indicates differential sensitivity for +\(\Delta\)conc at a given back-conc. A: response of a single ON ORN plotted as a function of +\(\Delta\)conc; trace, equal frequency line at 10 impulses/s. B: mean and its SD from pooled responses of 13 ON ORNs plotted as a function of +\(\Delta\)conc. Connecting the SDs above and below the means yields a band that contains 68% of the 65 points. Equation values are given in Table 1Aa.}\]
TABLE 1. Summary of data used to determine differential sensitivity and resolving power of the ON and OFF ORNs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Type of Olfactory Receptor Neuron</th>
<th>ON ORN</th>
<th>OFF ORN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Stimulus: change in odor concentration</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ORNs used for parabolic regressions</td>
<td>+Δconc</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Number of parabolic regressions with 5 points</td>
<td></td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Mean coefficient of determination, ( R^2 )</td>
<td></td>
<td>0.92 ± 0.11</td>
<td>0.98 ± 0.03</td>
</tr>
<tr>
<td><strong>Aa. Parabolic regression from pooled responses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back-conc 0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of parabolic regressions</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mean slope of parabolic regressions, (imp/s)/(Δ%)</td>
<td>0.22 ± 0.06</td>
<td>-0.47 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Mean deviations of responses, (imp/s)</td>
<td>4.45 ± 3.18</td>
<td>6.56 ± 5.83</td>
<td></td>
</tr>
<tr>
<td>Resolving power, %</td>
<td>56</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Back-conc 20%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of parabolic regressions</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mean slope of parabolic regressions, (imp/s)/(Δ%)</td>
<td>0.15 ± 0.05</td>
<td>-0.37 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Mean deviations of responses, (imp/s)</td>
<td>3.02 ± 2.63</td>
<td>6.35 ± 6.03</td>
<td></td>
</tr>
<tr>
<td>Resolving power, %</td>
<td>48</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Back-conc 40%</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Number of parabolic regressions</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mean slope of parabolic regressions, (imp/s)/(Δ%)</td>
<td>0.11 ± 0.01</td>
<td>-0.35 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Mean deviations of responses, (imp/s)</td>
<td>2.24 ± 1.74</td>
<td>8.26 ± 5.86</td>
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</tr>
<tr>
<td>Resolving power, %</td>
<td>46</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td><strong>Ab. Single parabolic regressions</strong></td>
<td></td>
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<tr>
<td>Back-conc 0%</td>
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<tr>
<td>Number of parabolic regressions</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Mean slope of parabolic regressions, (imp/s)/(Δ%)</td>
<td>0.17 ± 0.12</td>
<td>-0.14 ± 0.33</td>
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</tr>
<tr>
<td>Mean deviations of responses, (imp/s)</td>
<td>1.25 ± 1.11</td>
<td>2.44 ± 5.36</td>
<td></td>
</tr>
<tr>
<td>Resolving power, %</td>
<td>28</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Back-conc 20%</td>
<td></td>
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<tr>
<td>Number of parabolic regressions</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mean slope of parabolic regressions, (imp/s)/(Δ%)</td>
<td>0.15 ± 0.09</td>
<td>-0.33 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>Mean deviations of responses, (imp/s)</td>
<td>0.70 ± 0.57</td>
<td>2.03 ± 4.76</td>
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</tr>
<tr>
<td>Resolving power, %</td>
<td>17</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Back-conc 40%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Number of parabolic regressions</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Mean slope of parabolic regressions, (imp/s)/(Δ%)</td>
<td>0.11 ± 0.10</td>
<td>-0.32 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Mean deviations of responses, (imp/s)</td>
<td>0.68 ± 0.64</td>
<td>1.91 ± 3.29</td>
<td></td>
</tr>
<tr>
<td>Resolving power, %</td>
<td>24</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td><strong>B. Stimulus: steady background concentrations</strong></td>
<td></td>
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</tr>
<tr>
<td>ORNs used for parabolic regressions</td>
<td>24</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Mean values include ±SDs. back-conc, background concentration; +Δconc, concentration jumps; -Δconc, concentration drops.</td>
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for the two curves are low, 0.50 and 0.30, reflecting the great scatter of individual responses about the average curves.

Resolving power of the ON ORN to concentration jumps

Performance in a sensory cell is mainly its ability to discriminate. For discrimination, however, differential sensitivity is not enough. This is because differential sensitivity supposes a regression and the slope and height of a regression provide scant clues on the expanse of the cloud of points surrounding it. Here, attention is focused on a single pair of responses of a single ON ORN. How many percent must two \( +\Delta\text{conc} \) differ for it to be able to identify the larger of them with a given high degree of probability (e.g., 90%)? Resolving power addresses this question.

Resolving power for \( +\Delta\text{conc} \) was calculated separately at 0, 20, and 40% back-conc. For the procedure see METHODS. The basic data are given in Table 1A. According to our analysis, we hypothesize that at a back-conc of 0%, a pair of \( +\Delta\text{conc} \) must differ by 56% to achieve a 90% probability that a single ORN of average differential sensitivity will correctly identify the larger of them based on a single response to each. At a back-conc of 40%, the required difference is 46%.

The degree of scatter suggested by the bands in Fig. 4B, however, far exceeds that manifested by single curves, whose CD values lie between 0.85 and 0.99. The bands describe the ranges well enough in which 68% of the responses from the ON ORN population were found. Nonetheless, because the ranges reflect the variance in slope of the parabolic curves more than the scatter of points about them, the procedure considers just the scatter. Both scatter and slope are essential to determine resolving power. Pooling their effects, as done in Fig. 4B, only compounds the problem.

Eliminating the effect of slope variation on the estimate of the resolving power would require determining the deviation of the responses from their respective parabolic regressions. In this case, slope could have no effect unless the amount of deviation is itself slope dependent. We established that no connection exists between slope and deviation. It follows that the mean deviation of all points from their respective parabolic regressions and the variance of the deviations from its own corresponding slope would be characteristic not only of the population of parabolic regressions as a group. They would also be characteristic of an average parabola with an average slope. This is because independence of slope means that the scatter found at one slope could just as well be found at another.

The advantage of treating the series of responses individually is evident in Table 1Ab. The mean deviation of the responses from the parabolic regressions improved considerably (at 0% back-conc from 4.45 ± 3.18 to 1.25 ± 1.11 impulses/s and at 40% back-conc from 2.24 ± 1.74 to 0.68 ± 0.64 impulses/s). The hypothesized value of the resolving power is now 28% at the 0% back-conc and 24% at the 40% back-conc.

Responses of the OFF ORN to concentration drops

Figure 3F clearly shows that drops in odor concentration (\( -\Delta\text{conc} \)) influence impulse frequency (\( F \)) of the OFF ORN, but quite differently, depending on the background concentration (back-conc). The effect of back-conc on the response magnitude to \( -\Delta\text{conc} \) was studied as follows. Thirteen ORNs were first exposed for \( \geq 3 \) min to one of four different back-conc levels between 40 and 100% (i.e., at 40, 60, 80, and 100% back-conc) and then tested with \( -\Delta\text{conc} \) to various lower concentrations, in a total of 52 series. The stimulus paradigm was taken from the experiments on the ON ORN, but the data obtained differ from those of the ON ORN inasmuch as \( F \) of the OFF ORN rose as a progressive function rather than monotonic function of the size of the concentration change. The relationship is exemplified in Fig. 5A. The parabolic regressions approximating these functions tend to be steeper at lower back-conc, with the consequence that the rate with which \( F \) rises per unit increase in \( -\Delta\text{conc} \) is higher at lower back-conc. The equal-frequency line in Fig. 5A indicates that it takes a 64% \( -\Delta\text{conc} \) to elicit 30 impulses/s at 100% back-conc, but only a 26% \( -\Delta\text{conc} \) at 40% back-conc. For each parabola, the mean slope at the five points was computed and the mean ± SD determined. At back-conc levels of 60, 80, and 100%, for example, the values were \(-0.56 \pm 0.23, -0.53 \pm 0.15, \) and \(-0.47 \pm 0.25 \) (impulses/s)/\( \Delta\% \), respectively. (The negative values reflect the downward direction of concentration change, yielding a rise in impulse frequency, and specify the OFF ORN.) For either curve, the CD is 0.99.

The OFF ORN illustrated in Fig. 5A is typical of the 13 OFF ORNs examined in that the curves grow steeper with decreasing back-conc. It is also typical that the highest \( F \) values were obtained by presenting \( -\Delta\text{conc} \geq 40\%; \) this was possible only at back-conc beyond 60%. In Fig. 5B, the \( F \) values obtained for each combination of \( -\Delta\text{conc} \) and back-conc were pooled and the mean \( F \) and SD of the pooled responses plotted as a function of both parameters. Then, for each back-conc, the relationship between mean \( F \) values and \( +\Delta\text{conc} \) was approx-
imated by a single parabolic regression, and the mean slope and its SD for the five points determined. The mean slope values of the five points along the parabolic regression are $-0.47 \pm 0.17$ and $-0.35 \pm 0.11$ (impulses/s)/(Δ%) at the 60 and 100% back-conc, respectively (Table 1Ab). The bands connecting the SDs above and below the mean values reflect variance in both slope and scatter of points. Because of the combined effects of slope and scatter, the CD for the two curves is low, only 0.43.

Resolving power of the OFF ORN to concentration drops

The consequence of the large variance of individual points about the mean parabolic regressions (Fig. 5B) is evident when attempting to determine the resolving power of the OFF ORN for $-\Delta$conc based on pooled responses. The values hypothesized from our analysis are 35% at the 60% back-conc and 54% at the 100% back-conc (Table 1Aa). These are the differences that must separate two $-\Delta$conc if the larger is to be identified with 90% probability based on a single response of a single OFF ORN of average sensitivity to each of the two stimuli.

The great scatter displayed by the bands in Fig. 5B, however, far exceeds that found for single curves, whose CD values lie between 0.80 and 0.99. The scatter was improved when single ORNs were treated individually rather than by pooling data from all ORNs (Table 1Ab). Then the deviation of the responses became considerably smaller (at 60% back-conc from 6.56 ± 5.83 to 2.44 ± 5.36 impulses/s and at 100% back-conc from 8.26 ± 5.86 to 2.03 ± 4.76 impulses/s). The hypothesized value of the resolving power is now 40% at the 60% back-conc and 34% at the 100% back-conc.

Responses of the ON and OFF ORNs to background odor concentrations

As previously shown in Fig. 3, A and B, the ON and OFF ORNs continued to be active when a new concentration level was maintained and becomes a back-conc. In the face of constant back-conc, the discharge rates of both ORN types differ from those observed during and just after concentration changes. Moreover, the excitatory response of either ORN to a given concentration change is affected by the level of the back-conc. This situation raises the question as to whether the height of the back-conc is already apparent in $F$ and does the effects were not obvious. Both ORNs definitely react to rates of concentration change in the order of 10%/s (Tichy et al. 2005), but this is 500 times faster than 0.02%/s.

To examine the discharges under constant stimulus conditions, eight sensilla were exposed to three series of back-conc, each consisting of one upward and one downward sequence of the three back-conc levels tested in Fig. 3C. Each back-conc was maintained for 3 min before $F$ of both types of ORNs was determined, over the same period of 1 s. Without exception, all ON and OFF ORNs fired continuously at every back-conc, even at the 0% back-conc. In Fig. 6, A and B, the $F$ values obtained from the eight ON and OFF ORNs are plotted against the back-conc and the relation for each ORN was approximated by a parabolic regression (Fig. 5, A and B, dotted lines). The slope is steeper for the OFF than that for the ON ORN; that is, a given change in back-conc changes $F$ of the OFF more than that of the ON ORN. The mean value for the two slopes at the three back-conc levels is $0.03 \pm 0.02$ (impulses/s)/% for the ON and $-0.10 \pm 0.08$ (impulses/s)/% for the OFF ORN (Table 1Ba). Although the progression of $F$ with respect to back-conc is very orderly, the curves are flat and the CD poor; i.e., 0.30 for the ON and 0.49 for the OFF ORN. As such, the curves provide a rough estimate for the slope values and show that the differential sensitivity of the OFF ORN is greater, sign ignored, than that of the ON ORN.

Resolving power for background odor concentrations

Because the relationship of $F$ to back-conc in both types of ORNs was so orderly, the possibility of determining their resolving power appeared meaningful, despite the flat slope of the curves. Resolving power here refers to the difference that must separate two back-conc levels for one of them to be correctly identified with a given high degree of probability (e.g., 90%) as being greater than the other. The basis for the identification is a single response to each stimulus from a single ORN of average differential sensitivity. The demand placed on it is that the higher $F$ be associated with the larger...
stimulus (the higher back-conc for the ON ORN, the lower back-conc for the OFF ORN). Resolving power was calculated by the formula described in METHODS, with values for differential sensitivity and response reliability determined based on a group estimate by a single parabolic regression through all 144 points of the eight ON or OFF ORNs. Connecting the SDs above and below the mean value yields a band in which 68% of the 144 responses from the population of either type of ORN may be found (see Fig. 6, A and B). The basic data are summarized in Table 1B.a. The calculation gives a resolving power of >100% for the ON and 79% for the OFF ORNs. Using our analysis, we hypothesize that the ON ORN is not able to distinguish the level of different back-conc and the OFF ORN can distinguish only two levels of back-conc, i.e., very high from very low.

Nonetheless, because the bands in Fig. 6, A and B reflect the variance in slope of individual ORNs more than the scatter of points about them, the procedure is inadequate to the task of representing just the scatter. The first step in eliminating the effect of slope variation on the estimate of resolving power was to determine the deviation of individual responses from their individual parabolic regressions for each ORN. In a second step, all deviations were treated as though they belonged to a single ORN of average differential sensitivity (Table 1Bb). The deviation of the responses became smaller: in the ON ORN, the mean value was 0.69 instead of 3.41 impulses/s and in the OFF ORN, 0.94 instead of 1.43 impulses/s. The hypothesized values of the resolving power are now 66% for the ON and 21% for the OFF ORN. Accordingly, the ON ORN can distinguish very high back-conc from very low back-conc and the OFF ORN five different levels of back-conc.

DISCUSSION

In the present study we report the response characteristics of the ON and OFF ORNs that occur together in a specific type of olfactory sensillum on the cockroach antenna. In both types of ORNs, the discharge rate is affected simultaneously by the extent of change in odor concentration and by the back-conc level from which the change was initiated. Furthermore, under constant back-conc, the ON and OFF ORNs exhibit a continuous discharge with a constant frequency that depends on the back-conc level. The odor of lemon oil—highly effective in exciting antennal ORNs and antennal lobe neurons—was used for stimulation (Boeckh 1974; Sass 1978; Selzer 1981, 1984; Zeiner and Tichy 2000).

Classification of the cockroach olfactory sensilla

Schaller (1978) defined and classified the antennal sensilla of cockroaches based on physiologically relevant criteria such as wall structures, presence and positions of pores, and the number of receptor neurons. She identified terminal-pore, non-pore, and wall-pore sensilla and distinguished three types of single-walled sensilla (type A constitute about 8% of the sensillum population, type B about 54%, and type C about 6%) and two types of double-walled sensilla (types A and B represented about 8% of the sensillum population).

These types of wall-pore sensilla were then matched with physiological observations by testing a large number of odors such as pheromones, fruits, meat, bread, and cheese and also chemically pure substances that are emitted by these odor sources (i.e., pentanol, hexanol, octanol, alcohol-terpene, and butric acid; Boeckh and Ernst 1987; Sass 1972, 1976, 1978; Schaller 1978). In contrast to many insect species, where the ORNs for pheromones and food odors are located in different morphological types of sensilla, in the cockroach they both occur in the single-walled type B sensilla. Furthermore, the nonpheromonal ORNs respond to broadly overlapping spectra of natural odors and synthetic compounds. When classified according to the most potent stimulatory compound (Boeckh and Ernst 1987; Sass 1972, 1976, 1978), the best stimulus in one type appeared as the second-best or third-best stimulus in another type. This variation in the best stimulus from type to type indicates that the respective choice of concentration could yield a different classification. Because of the poor selectivity of the response spectra, Sass (1978) classified the ORNs by considering three modes of response (no response, weak, or strong excitatory response) to a selected group of natural food odors (banana, apple, lemon, orange, bread, meat, and cheese). Lemon produced strong excitatory responses in two classes of ORNs and somewhat weaker responses in nine additional classes. The first two lemon classes, however, match with the octanol-best and alcohol-terpene-best types (Sass 1976, 1978).

Both functional types have been assigned as single-walled type B sensilla, which represent about 54% of the sensillum population. Since the pheromone ORNs were also found in this sensillum type, it may be that less than half of them (27%) respond to lemon odor. This is equivalent to four times the numbers of the single-walled type C sensilla (6%), which house the ON and OFF ORNs.

The designation ON and OFF ORNs refers to their opposite responses to changes in the concentration of the lemon odor. Although not quantified, the same opposite responses were obtained to odors of lemon, orange, apple, and banana. The odors emitted from baked bread had a weak effect, whereas meat and cheese elicited no response (Hinterwirth et al. 2004; Tichy et al. 2005).

Occurrence of ON and OFF ORNs in single sensilla

The ON and OFF responses have been recognized because both an increase and a decrease in the odor concentration produce striking discharge rates. By testing different levels of back-conc, the optimal initial stimulus condition for eliciting excitation in the OFF ORNs was found (Hinterwirth et al. 2004). A major advantage was that the activity of the ON and OFF ORNs can be recorded simultaneously with the same electrode. This removed any doubt that the two types of ORNs provide a means for transmitting information about both concentration increments and concentration decrements with excitation.

Since concentration increments and decrements do not occur physically at the same time in the same place, the polarity of the responses of the ORNs to changes in odor concentration strikingly resembles complementary pairs of electronic amplifiers. By using a “push–pull” arrangement, each amplifies the opposite halves of the input signal, which gives excellent efficiency. The effective transfer for either sign of concentration change by a dual system of ON and OFF ORNs will profit from a single receptive field by combining them in the same sensilla.
Phasic and tonic components in the ON and OFF responses

The ON and OFF ORNs display large phasic responses to the transient aspect of the odor stimuli. By adapting partially to the maintained aspect of the odor stimulus, the ORNs discharge continuously until the end of stimulation. Adaptation not only accentuates the response to concentration increments and decrements, but also indicates the presence of a back-conc level on which new changes are superimposed. In this way incomplete adaptation allows signaling concentration changes against the back-conc and provides information about the duration of the steadily acting odor concentration. This also has profound implications on the detection of temporally structured odor signals. Odor patterns have similarities and differences, such as equal durations but different intervals or equal intervals but different durations. Moreover, many ON and OFF durations last hundreds of milliseconds and it is unclear how the nervous system could measure such long times in the different patterns. One may suggest that earlier and later durations are stored and compared, but how the insect nervous system could perform this task is unknown. Another possibility is a neuronal network, in which each pattern evokes activity in a unique set of neurons, whose activity does not change if the pattern is not changing; again, this model lacks experimental evidence. A direct transduction of the ON and OFF durations into the duration of the ORN’s discharge evoked by them enables detection of temporal odor patterns without explicit knowledge of time. This is realized by the ON and OFF ORNs, which not only signal the arrival and termination of the odor stimulus but also its duration and spacing by specialized ORNs.

ON and OFF responses to changes in odor concentration

As might be expected, the greater the concentration change, the greater the magnitude of the response, depending of course on the direction of change and type of ORN. In the ON ORN, the parabolic regressions approximating this function tend to be steeper where the drops are smaller and to flatten as they become greater. Conversely, in the OFF ORN, the parabolic regressions are flatter where the drops are smaller and steeper as they become greater. Thus the ON ORN is more sensitive to smaller jumps than to larger ones and the OFF ORN is more sensitive to larger than to smaller drops. Importantly, the functions are not the same at all back-conc levels. In both types of ORNs, the response to the same concentration change varies with the back-conc. When superimposed on rising levels of back-conc, the ON-ORN’s response to concentration jumps and the OFF-ORN’s response to concentration drops are decreased. Thus the responses of the ON and the OFF ORNs are desensitized at higher concentration levels (Dolzer et al. 2003; Li 1990).

Although the back-conc has opposite effects on the response magnitude of the ON and OFF ORNs, it affects their differential sensitivities for concentration changes in a similar manner. An increase in back-conc depresses the differential sensitivities of both types of ORNs. Thus to elicit a given response magnitude in both types of ORNs the extent of the concentration change must increase with increasing back-conc. However, the reliability in signaling concentration changes is affected differentially in the ON and OFF ORNs. Although in the ON ORN the scatter of individual responses about the parabolic function tends to become smaller with increasing back-conc, in the OFF ORN it tends to become larger. Using a theoretical model of resolving power, we obtained that the ability of the ON ORN to distinguish concentration changes improves with increasing back-conc level from 56 to 46%, but deteriorates in the OFF ORN from 35 to 54%.

Stimulus information on which the cockroach’s discrimination is based depends on centrally integrating that information conveyed by single ORNs. The most straightforward interpretation of our data is that the stimulus information conveyed by numerous ORNs engaged by the stimulus is combined centrally by a simple additive or averaging process and that each ORN’s response is given equal weighting in the integrative process. Our analysis indicates that this simple integrative process retains stimulus information to account for a rough resolution of incremental changes in odor concentration, even when superimposed on increasing levels of back-conc. If these conditions do not pertain, the central process of combining the responses of individual ORNs must be selective so that those ORNs that signal the greatest stimulus information contribute maximally to the discriminative process and, less responsive, contribute less to the process. The present analysis demonstrates that the ORNs differ in their susceptibility to concentration changes but that the different slopes were not simply due to response variability. That is, the functions obtained from a given back-conc level did not display any one of a large number of different slopes. Variability occurred across the different ORNs. Consequently, the distribution of slopes from a given back-conc level was not due to an individual ORN’s variability with respect to a given concentration series but to the fact that individual ORNs differed in their responsiveness to concentration changes. However, when treating the series of responses of the different ORNs individually and pooling the data to provide a group estimate for all ON and OFF ORNs, the effect of the back-conc level on the differential sensitivities disappeared but the resolving power for concentration changes improved. Our analysis indicates that, despite the variability among the ON and OFF ORNs, the animals optimally discriminate concentration changes if the central integration of the responses is selective.

Note that the two processes that the cockroach’s brain might use to combine the sensory inputs of simultaneously responding ORNs—the equal weighting and the optimal weighting of the responses of individual ORNs—are each logically simple and estimated based on the actual recorded action potentials. Since the response to the same change in odor concentration varies with the concentration level from which the change is initiated, each response is ambiguous, not with regard to the direction of the concentration change but with regard to its extent. Each response can be elicited by various combinations of concentration change and back-conc. The individual ORN therefore has limited ability to distinguish the two components of the odor stimulus. Nevertheless, several ORNs with different response characteristics taken individually and compared do seem to enable quantitatively separating both components. By simultaneously using the output of different ON ORNs, the number of possible combinations satisfying the same response should be reduced (if the irregularities of the curves are not significant). Taking the OFF ORNs additionally into account should further reduce errors from response variation.
ON and OFF responses to the background concentration

The tonic responses of the ORNs display a slight dependence on the back-conc. Based on a theoretical model of resolving power, the ability to distinguish back-conc is better in the OFF than that in the ON ORN due to the greater differential sensitivity and reliability of the former. Although the OFF ORN can discriminate five concentration levels within the whole range, the ON ORN can distinguish only high from low concentrations. It is tempting to speculate on the consequences of the cockroach’s resolving power for constant odor concentrations. Does it reflect conditions regularly encountered by the insect?

Comparison

The cockroach’s ON ORN is not the only chemoreceptor in which the response to concentration jumps decreases with increasing back-conc. The ammonia receptor cells of the walking legs of the lobster Homarus americanus (Borroni and Atema 1988) display progressively weaker responses to changes in ammonia concentration the higher the background ammonia concentration level. As in the cockroach’s ON ORN, each unit increase in the back-conc level tends to decrease the discharge to all ammonia concentrations by an approximately constant amount. The result is a parallel shift of the stimulus–response function to the right along the abscissa. This indicates that, on average, for a given increase in back-conc the decrease in responsiveness to a series of ammonia concentrations is constant. Each response can be elicited by a variety of combinations of back-conc levels and changes in ammonia concentrations. Quite similar to the cockroach’s ON ORN, the ammonia cells function as detectors for relative rather than absolute concentration changes.

With respect to the cockroach’s OFF ORN, excitatory responses to decreasing odor concentration are unique to date. Other terrestrial arthropods remain to be examined in this regard. In the antennal lobe of the locust, however, Mazor and Laurent (2005) observed a specific type of projection neuron (PN) that produces excitatory OFF responses. This OFF PN fell silent when the antennae are exposed to cis-3-hexen-1-ol. The offset of odor presentation elicits a sharp rise in the discharge; this is followed by a gradual decline to a constant level (PN4 in Fig. 1B; Mazor and Laurent 2005). The origin of these OFF responses remains to be determined, i.e., whether they are generated by olfactory processing in the antennal lobe or go back to a yet undescribed peripheral OFF ORN on the locust antennae.

Functional asymmetries

Under constant odor concentrations, the OFF ORN is better able to resolve small differences than the ON ORN. This reflects the greater differential sensitivity and reliability of the former. In its response to concentration changes, the OFF ORN has a lower differential sensitivity and reliability than the ON ORN. Thus the ON ORN can discriminate concentration jumps better than the OFF ORN can distinguish concentration drops. During concentration changes, the higher impulse frequency of the OFF ORN is less accurate than the lower impulse frequency of the ON ORN. The two ORNs are thus not a symmetrically responding set of receptor neurons, revealing a fundamental nonlinearity in odor detection.

This asymmetry may compensate for a diminishing differential sensitivity for concentration changes with rising back-conc levels. As shown here, the impulse frequency of both ORN types is simultaneously affected by two independent parameters: the extent of concentration change and the back-conc level. Jumps in concentration elicit strong responses in the ON ORNs, but the response to a given jump tends to be lower the higher the back-conc level (Fig. 7A). Similarly, the response of the OFF ORN is high when the drop is large, but the response to a given drop tends to be lower the higher the back-conc level (Fig. 7B). At the same time the OFF ORN has a larger frequency range, i.e., it responds with higher frequency values to a drop than the ON ORN to an equivalent jump.

A progressively compressed scaling as described by the decreasing slope of the stimulus–response functions with increasing back-conc level has certain advantages: it optimizes sensitivity for the low concentration range without sacrificing the range of operation and without unduly extending the measuring scale. A high back-conc level, however, may convey a vital message. It signals proximity to the odor source. Nonetheless, when the back-conc level is rising, concentration jumps become less effective in eliciting excitation in the ON ORN. At the same time, when the cockroach turns to an area where the concentration is lower than the background, the OFF ORN will discharge with high rates, which are even higher than those of the ON ORN to concentration jumps at the same background concentration (Fig. 7C). When searching for the
odor source, the cockroach should balance between the discharge rates of the ON and OFF ORNs. Clearly, high discharge rates of the ON ORN signal the direction toward the odor source. Low discharge rates will also do so, provided that a change in the course elicits a high discharge rate in the OFF ORN. Strong responses of the OFF ORN will serve as an early message that the concentration is falling: turn back!

**REFERENCES**


