Chapter 5

Background odour induces slight adaptation and sensitization of olfactory receptors in the antennae of *Musca domestica* L.

Summary

The presence of background odour affects the sensitivity of the antennal olfactory system of houseflies to new pulses of odour only to a small extent. We show that cross-adaptation and cross-potentiation between a background odour of 1-octen-3-ol and pulses of 1-octen-3-ol, 2-pentanone and R-limonene can occur, confirming that olfactory receptor cells are sensitive to different odours. Background odour can increase the responses to low concentration odour pulses and decrease the responses to higher concentration odour pulses. It is suggested that background odour has a larger effect on olfactory receptor cells that respond with a tonic increase of spike frequency, giving information about the level of odour concentration, i.e. the “static” environment. Cells that respond in a phasic way only provide information on the dynamics of the olfactory environment.
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Introduction

The housefly, *Musca domestica*, is one of the most familiar nuisance pests of human and livestock habitations and constitutes a major problem in a variety of industries, such as cattle and poultry farms and food processing industries (Hansens, 1963). Flying between various food sources, houseflies may be vectors of many diseases such as dysentery, gasteroenteritis, cholera and tuberculosis (Grübel *et al.*, 1997; Tan *et al.*, 1997). Therefore, methods are developed to control housefly populations. Some methods of control are based on olfactory cues, luring houseflies into traps with attractive odours, or repelling them with unpleasant odours. To identify chemical substances that can be used as baits or repellents for houseflies, several behavioural and electrophysiological studies have been done (Richardson and Richardson, 1922; Wieting and Hoskins, 1939; Dethier *et al.*, 1952; Frishman and Matthysse, 1966; Mulla *et al.*, 1977; Warnes and Finlayson, 1986; Cossé and Baker, 1996). Most of these studies were performed in the laboratory in which test chemicals were added to clean air to observe the responses of the flies to these chemicals. However, in nature, air is always loaded with some ambient background odour. For attractants or repellents to be effective, the flies have to be able to distinguish these chemicals from the ambient odours.

In this study we investigate the influence of ambient odours on the electrophysiological responses to some attractive and repellent odours. We compare the sensitivities of antennal olfactory cells to odours added to clean air and applied in the presence of either a synthetic (1-octen-3-ol) or a natural background odour (chicken manure). We recorded electroantennograms (EAGs) and spike responses of individual antennal cells on stimulation with 1-octen-3-ol, 2-pentanone and R-limonene. 1-Octen-3-ol is known to be an attractant for several dipteran species (Hall *et al.*, 1984) and R-limonene appeared to be repellent to houseflies (dr. Moskal, Denka International, pers. comm.). 2-Pentanone was found to be electrophysiologically active in previous studies (Chapters 3, 4).

Materials and Methods

*Insects.* Pupae of a *Musca domestica* WHO strain Ij2 were obtained from the Danish Pest Infestation Laboratory (Lyngby, Denmark). Cultures of the strain were kept in the laboratory at 25°C and 75% RH. Larvae were reared in an aqueous jelly of agar, yeast and skim milk powder (1:5:5 by weight), and allowed to pupate in wood curls on top of the jelly. Flies were fed a mixture of milk powder, sugar and autolysed yeast (5:5:1 by
weight) and had access to tap water. Experimental flies were 4-20 days old (mature).

**Electrophysiology.** An intact fly was immobilized in a plastic pipette, with its head protruding from the tip, so that its antennae were accessible for recording (Den Otter et al., 1988). Glass micropipette/Ag-AgCl electrodes filled with Beadle-Ephrussi saline were used. For both electro-antennogram (EAG) and single cell (SC) recordings the tip (ca. 25 µm) of the indifferent glass electrode was inserted into the head of the fly between the eyes, near the base of the antennae. EAG recordings were made by placing the tip (25-100 µm) of a recording electrode over the distal end of the antenna. Single cell recordings were obtained using the surface-contact technique (Den Otter et al., 1980). By gently pressing the tip of the recording electrode (< 5 µm) against the cuticle of the funiculus, spikes from individual receptor cells could be recorded. When electrical contact with the antenna was made, the preparation was left for 5 minutes before starting an experiment.

The electrodes were connected to a high-impedance AC/DC amplifier (Syntech, Hilversum, The Netherlands). The EAG-signal was directly fed into a computer. The SC-signal was displayed on an oscilloscope, made audible via a speaker and stored on tape with a DAT-recorder for later analysis.

**Stimuli.** Chemicals used to prepare test stimuli were obtained from Fluka (1-octen-3-ol, 2-pentanone, >98%) and Denka (R-limonene, >98%). The chemicals were dissolved in silicon oil, 0.4 g/ml solutions and 4 decadie dilutions were made. Aliquots of 25 µl of each solution were pipetted onto a 6x35 mm² filter paper in a Pasteur pipette. The pipettes served as odour cartridges and thus contained 0.001, 0.01, 0.1, 1 or 10 mg of the chemicals.

A constant flow (3 ml/s: A) of charcoal-filtered, humidified air was led through a clean empty bottle or through a bottle with the background odour source. The latter bottle contained 5 g fresh chicken manure, or a filter paper loaded with 250 µl silicon oil containing 1, 10 or 100 mg 1-octen-3-ol, or loaded with 1250 µl (1000 mg) pure 1-octen-3-ol. This clean or background odour-loaded air was added to an airstream (10 ml/s: B) continuously passing over the antennae through a stainless steel tube (i.d. 7 mm), the outlet of which was about 5 mm from the preparation. Stimulation with test odour occurred by injecting, in 0.2 s, 2 ml vapour (10 ml/s: C) from an odour-loaded pipette into the airstream through an aperture (3 mm diameter) in the tube, 8 cm from its outlet. The complementary airstream (B) was switched off during the stimulus by a stimulus controller (Syntech) to maintain the total flow over the antennae at 13 ml/s. Concentrations of the background odour in the bottles and the test stimuli in the odour
cartridges were determined using a Shimazu GC-7A gas chromatograph, and the resulting diluted concentrations in the air at the site of the antennae were calculated. Odours were presented at 60 s intervals in random sequence, in (1) clean air, (2) background odour-loaded air and (3) again in clean air. Between (1) and (2) the preparation was flushed with background odour for at least 15 min and between (2) and (3) with clean air for at least 15 min.

**Analysis.** EAGs were analysed using the software package EAG V2.6c (Syntech). To compare the EAG-responses of different experiments, the EAG amplitudes were normalized. First, the responses were calculated as a fraction of the mean response to 10 mg 1-octen-3-ol of each experiment; then, this fraction was multiplied by the mean EAG-response (in mV) to 10 mg 1-octen-3-ol of all experiments.

**Figure 1.** EAG dose-response curves of 1-octen-3-ol when flushed with clean air (‘before’), with background 1-octen-3-ol odour (‘test’) and again with clean air (‘after’). The concentrations of background 1-octen-3-ol are indicated in each graph. Error bars represent SEM. Significant differences (p < 0.05) between responses during background odour and clean air ‘before’ (*) and ‘after’ (#) are indicated.

The action potentials were analyzed using the software package Autospike™
(Syntech). Spikes from different cells were distinguished by their amplitudes. It was uncertain at what moment the bulk of the stimulus reached the recording site after injection. Therefore, the response magnitude was calculated from the maximum number of spikes in a sliding 0.1 s period of the response, and expressed as spikes/s. The mean non-stimulated activity (spikes/s) during the 3 s prior to the beginning of the stimulation was determined and subtracted.

Only cells that responded to the test odours in a dose-dependent manner were used for further analysis. A cell was considered to respond in a dose-dependent manner when the responses to at least the higher two doses of a chemical were higher than the responses to the lower doses applied.

**Results**

Figures 1, 2 and 3 show the EAG dose-response series of the three test odours when applied in clean air and in four different background concentrations of 1-octen-3-ol. For

![Figure 2](image)

**Figure 2.** Dose-response curves of 2-pentanone when flushed with clean air (‘before’), with background 1-octen-3-ol odour (‘test’) and again with clean air (‘after’). See Fig. 1 for details. a quick screening of the effect of a background odour on the sensitivity of the antennal
olfactory system, Repeated Measurements ANOVA tests were performed. No significant effects of the two lower background concentrations of $2.2 \times 10^{-8}$ and $9.5 \times 10^{-8}$ M 1-octen-3-ol were found. In both absence and presence of these concentrations the responses to the test odours were the same (Figs 1, 2, 3A, B). When, however, background concentrations of $1.7 \times 10^{-7}$ and $2.4 \times 10^{-7}$ M 1-octen-3-ol were present, the shape of the dose-response curves differed significantly from those in clean air. Repeated Measurements ANOVA showed significant interactions of dose with presence or absence of $1.7 \times 10^{-7}$ M 1-octen-3-ol background odour for 1-octen-3-ol (p = 0.008, F(8, 10) = 2.9) and for 2-pentanone (p = 0.001, F(8, 10) = 4.0) and with presence or absence of $2.4 \times 10^{-7}$ M 1-octen-3-ol background odour for 1-octen-3-ol (p < 0.001, F(8, 6) = 5.6) and for R-limonene (p = 0.014, F(8, 6) = 2.9). Therefore we considered the effects of these background concentrations in more detail.

A background concentration of $1.7 \times 10^{-7}$ M 1-octen-3-ol (Figs 1, 2, 3C) increased the responses to the lower two or three concentrations of 1-octen-3-ol and R-limonene

![Figure 3. Dose-response curves of R-limonene when flushed with clean air (‘before’), with background 1-octen-3-ol odour (‘test’) and again with clean air (‘after’). See Fig. 1 for details.](image_url)
Effect of background odour on olfactory sensitivity

Figure 4. Example of a spike response to manure. Horizontal bar indicates the duration of the stimulus (0.5 s).

compared to the responses in clean air. In contrast to this, the background concentration decreased the responses to the higher concentrations of 1-octen-3-ol and 2-pentanone. After having switched back to clean air in the ambient, some recovery could be seen, the responses to the test substances becoming higher again. Interestingly, $1.7 \times 10^{-7}$ M background 1-octen-3-ol did not inhibit the responses to the higher concentrations of R-limonene.

Flushing the preparation with a $2.4 \times 10^{-7}$ M 1-octen-3-ol background concentration decreased the EAG responses to the higher two concentrations of 1-octen-3-ol and the highest concentration R-limonene (Figs 1, 2, 3D). After switching back to clean air, no significant recovery of the EAG responses occurred.

In the single cell experiments we only tested the effects of the two intermediate concentrations of background 1-octen-3-ol: $9.5 \times 10^{-8}$ M and $1.7 \times 10^{-7}$ M. In addition, we used the odour of chicken manure as a natural background odour. This odour had been proven to evoke responses in antennal cells (Fig. 4).

It appeared that on application of background 1-octen-3-ol two different types of single cell responses could be observed: some cells showed a short phasic, others a sustained tonic increase of spike activity (Fig. 5A, B). In the latter cells, spike activity returned to the initial level after switching back to clean air (Fig. 5C). No changes in spike frequency were observed in phasic cells while switching back to clean air. Some cells responding to 1-octen-3-ol also responded to the test substances 2-pentanone and R-limonene. Some cells were only sensitive to 1-octen-3-ol and 2-pentanone, others to 1-octen-3-ol and R-limonene, and some cells responded to 1-octen-3-ol only.

Six cells showed a tonic response and 2 cells a phasic response during continuous stimulation with $9.5 \times 10^{-8}$ M 1-octen-3-ol background odour. The initial non-stimulated activity of all 8 cells did not differ (tonic: $9.0 \pm 6.6$ spikes/s; phasic: $9.1 \pm 0.7$ spikes/s, Table 1). During the presence of the background odour, the spike frequency of the tonic cells increased significantly to $20.1 \pm 9.0$ spikes/s ($p = 0.03$, Paired Wilcoxon test),
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Figure 5. Examples of 50 s spike activity during onset and offset of background odour. Horizontal bars indicate the presence of background odour. Each column in the lower graphs shows the number of spikes in the corresponding second. A) Cell responding with a short phasic response to the onset of 1.7 x 10^{-7} M 1-octen-3-ol. B) Cell responding to the onset of 9.5 x 10^{-8} M 1-octen-3-ol with a tonic response, that lasted all the time (0.5 hr) background odour was present, after which, C) the spike frequency returned to the initial level.
whereas the spike frequency of the phasic cells stayed at 7.3 ± 4.8 spikes/s after a short burst at the onset of the background odour.

On continuous application of $1.7 \cdot 10^{-7}$ M 1-octen-3-ol or the odour of chicken manure as background odour, no tonic responses were observed: all cells tested showed a phasic response to these odours, after which the spike frequencies returned to initial values (Table 1).

Figure 6. Single cell measured dose-response curves of 1-octen-3-ol (A, B), 2-pentanone (C, D) and R-limonene (E, F) when flushed with clean air (‘before’), with background 1-octen-3-ol odour (‘test’) and again with clean air (‘after’). See Fig. 1 for details.
**Table 1.** Average spike frequencies ± STD (spikes/s) of tonically and phasically responding cells in clean air (‘before’), during background odour (‘during’), and after switching off the background odour, again in clean air (‘after’). $1o3 = 1$-octen-3-ol. The number of cells tested is indicated between brackets.

<table>
<thead>
<tr>
<th>Background odour</th>
<th>before</th>
<th>during</th>
<th>after</th>
</tr>
</thead>
<tbody>
<tr>
<td>$9.5 \cdot 10^{-8}$ M $1o3$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tonic cells (6)</td>
<td>$9.0 \pm 6.6^*$</td>
<td>$20.1 \pm 9.0^*$</td>
<td>$14.3 \pm 9.2$</td>
</tr>
<tr>
<td>phasic cells (2)</td>
<td>$9.1 \pm 0.7$</td>
<td>$7.3 \pm 4.8$</td>
<td>$7.4 \pm 3.9$</td>
</tr>
<tr>
<td>$1.7 \cdot 10^{-7}$ M $1o3$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phasic cells (8)</td>
<td>$11.6 \pm 6.5$</td>
<td>$8.9 \pm 6.5$</td>
<td>$9.0 \pm 7.4$</td>
</tr>
<tr>
<td>$5$ g manure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phasic cells (12)</td>
<td>$8.8 \pm 8.0$</td>
<td>$8.8 \pm 6.8$</td>
<td>$8.4 \pm 6.7$</td>
</tr>
</tbody>
</table>

$^*$ indicates a significant difference ($P = 0.031$, paired Wilcoxon test).

**Figure 7.** Dose-response curves of $1$-octen-3-ol (A), $2$-pentanone (B) and $R$-limonene (C) when flushed with clean air (‘before’), the odour of $5$ g chicken manure (‘test’) and again with clean air (‘after’). See Fig. 1 for details.
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The $9.5 \times 10^{-8}$ M 1-octen-3-ol background odour affected the responses of the two phasic cells to the test 1-octen-3-ol in a comparable way as the six tonic cells: by lowering the responses of the cells to the higher two test doses of 1-octen-3-ol compared to the responses in clean air. The two phasic cells did not respond to 2-pentanone or R-limonene. Figure 6 shows the responses of the tonic cells only.

Repeated Measurements Anova tests indicated no significant differences between the dose response series in clean air and during background odour for any test odour (Figs 6, 7). An inhibitory effect of the $9.5 \times 10^{-8}$ M 1-octen-3-ol on the responses to the higher test doses can be seen, but this was not significant for any single concentration in clean air compared with the response in background odour. The $1.7 \times 10^{-7}$ M 1-octen-3-ol and the odour of chicken manure as background odour did not inhibit the responses to the test odours.

Discussion

The presence of background odour may affect the sensitivities to additional pulses of odour. This effect of background odour was more pronounced in the EAG experiments than in the single cell experiments. The EAG experiments showed no effect of the $2.2 \times 10^{-8}$ and $9.5 \times 10^{-8}$ M background 1-octen-3-ol on the responses to the test odours 1-octen-3-ol, 2-pentanone and R-limonene. However, in the presence of $1.7 \times 10^{-7}$ M background 1-octen-3-ol the responses to the lower doses of 1-octen-3-ol and R-limonene appeared to be enhanced, whereas the responses to some of the higher doses of 1-octen-3-ol and R-limonene seemed to be inhibited, compared to the responses in clean air. During stimulation with $2.4 \times 10^{-7}$ M background 1-octen-3-ol the responses were lower than before in clean air and did not reach their original level after the background odour was switched off and replaced by clean air again.

These effects may be explained by considering the stimulus transduction processes of odours as summarized by Kaissling (1996). Odour molecules diffuse through the cuticular pores (see Chapter 2) into the sensillum lymph. Odorant binding proteins (OBPs) may transport the odour molecules through the sensillum lymph towards the odorant receptors in the dendrites of the olfactory cells (Vogt and Riddiford, 1986; Van den Berg and Ziegelberger, 1991). Upon binding of the odorant-OBP complex to the receptors, the latter are activated. Then, ion channels open, depolarizing the dendrite of the olfactory cell. The ion channels may be directly coupled to the receptor molecules, or may be activated via the IP$_3$ or cAMP second messenger system (Krieger et al., 1997). Upon depolarization of the dendrite, the soma of the receptor cell generates action potentials that are sent via the axon to the brain. For deactivation of the odour, it was proposed that the odorant-OBP complex is rapidly oxidized, possibly by the receptor (Ziegelberger,
This oxidized odorant-OBP complex is no longer able to stimulate receptor molecules. Finally, the odour is degraded by enzymes (Vogt and Riddiford, 1986; Rybczynski et al., 1990).

The effect of potentiation or facilitation found in our study at low doses of test odour may be the result of the fact that in the presence of background odour the olfactory cell membrane is more depolarized and is, thus, discharging action potentials in a higher frequency than in the non-stimulated situation. The extra stimulus of test odour will increase the depolarization and spike frequency even more. When a high-dose odour puff is presented in high-dose background odour, the response to this puff on top of the response to background odour is lower than in clean air. Competition for odorant binding proteins and receptor sites may occur. The capacity of receptor sites approaches saturation and excess odour molecules might be shunted towards degrading enzymes and away from receptors. Therefore, at high test concentrations, the response may be lower during the presence of background odour than in clean air.

On continuous stimulation with $9.5 \times 10^{-8}$ M background 1-octen-3-ol odour, some cells responded with a tonic increase in spike frequency, whereas others showed a short phasic increase of spike frequency at the onset of the background odour, quickly returning to the initial spike frequency. These two types of responses were also found in other insect species, e.g. moths (Almaas et al., 1991; Heinbockel and Kaissling, 1996) and tsetse flies (Den Otter and Van der Goes van Naters, 1992; Voskamp et al., 1998). The cells showing phasic responses and quickly adapting to odour may be considered to respond to the dynamic increase of odour concentration. The cells that keep a tonic higher spike frequency during the stimulus provide information on both the dynamic and the static phase of odour stimulation. Although we did not find significant differences between the phasic and tonic cells investigated, the sensitivity to new odour puffs may be more affected by background odour in the latter type of cells, as in these cells the continuous response to the background odour may interact with the responses to new odours. It was shown in tsetse flies that the sensitivity and temporal resolution in tonic cells was lower than in phasic cells (Voskamp et al., 1998). The presence of both phasic and tonic responding cells increases the temporal and dynamic range of the olfactory system of the animal.

Our experiments showed that the presence of background odour of 1-octen-3-ol can affect the EAG-responses to test odour pulses of 1-octen-3-ol, 2-pentanone and, to a lesser extent, R-limonene. Therefore, olfactory cells are present that are sensitive to 1-octen-3-ol, as well as 2-pentanone and R-limonene, as was found in several single cell experiments. Two possible models explain this multiple sensitivity: either the three compounds share one receptor in the olfactory cell membrane, or, alternatively, they may
bind to different receptor sites that activate one shared intracellular signal transduction cascade. Both models can explain cross-adaptation and cross-potentiation (Derby et al., 1991). In the antennae the sensory cell population may consists of a mixed population of cells with unspecific receptors and cells with several different specific receptors, sharing the same intracellular signal transduction pathway (Daniel and Derby, 1991).

We tested chicken manure as a natural background odour. However, the concentration of this complex odour mixture is difficult to estimate, and no information about the concentration in chicken farms was present. If the responses in the natural habitat of the flies are comparable to those found in this study, the presence of a large odour concentration in habitats as, for instance, chicken farms, may not affect the sensitivity of the olfactory system of flies to new odour pulses to a large extent. Therefore, using baits, even containing odour of manure, may be effective in luring animals to control fly populations in smelly, odour-loaded environments.

References


