INTRODUCTION

Time-dependent properties of chemoreception are crucially important in many situations, but poorly understood. Examples of olfactory dynamic sensitivity include the dependence of moths and mosquitoes on the temporal structures of pheromone and CO₂ plumes, respectively (Geier et al. 1999; Justus et al. 2005). Dynamic characterization can also provide tools for dissecting functional components of chemoreceptor pathways, both peripherally and centrally, and for quantifying olfactory information transmission.

Two major practical obstacles have limited studies of chemoreceptor dynamics: the difficulty of rapidly controlling the concentration of an odorant or gustatory chemical at the sensory receptor surface and the difficulty of measuring that concentration. A significant advance has been provided by photoionization detectors (PIDs) that can measure gas concentrations over wide ranges with millisecond resolution. PIDs have already shown the limitations of traditional odorant stimulation protocols (Vetter et al. 2006) and allowed dynamic electroantennogram measurements of moths with pheromones (Justus et al. 2005) and Drosophila with fruit odors (Schuckel et al. 2008). Dynamic control of odorant stimulation has progressed to allow both continuously and digitally modulated chemical concentration (French and Meisner 2007; Schuckel and French 2008).

Drosophila olfactory sensilla are on the third antennal segment (Vosshall and Stocker 2007). Each contains one to four bipolar sensory neurons. Odor-detecting response properties of individual neurons are controlled by odorant receptor molecules (ORs) expressed in their dendrites (Hallem et al. 2004; Hiroi et al. 2008). Recent evidence suggests that ORs are direct, odorant-activated cation channels, although second messenger transmission to ion channels may also exist (Sato et al. 2008; Wicher et al. 2008).

Both excitatory and inhibitory responses have been seen in insect olfaction. Some cockroach sensilla contain two neurons that responded with opposite polarity to lemon oil, although it is not clear which compounds in the oil caused the two responses (Tichy et al. 2005). A few Manduca sexta olfactory sensilla gave inhibitory responses to a range of odors (Shields and Hildebrand 2001). Drosophila basiconic and coeloconic sensilla were predominantly excited by fruit odors, but some neurons were slightly inhibited, whereas certain terpenes inhibited responses to other excitatory compounds (de Bruyne et al. 2001; Hallem et al. 2004; Yao et al. 2005). Drosophila trichoid sensilla were excited by pheromones (van der Goes van Naters and Carlson 2007) but inhibited by fruit odors (Hallem et al. 2004).

Dynamic characterization of insect olfactory receptors has primarily concentrated on maximum frequency discrimination using pulsatile stimuli (Barrozo and Kaissling 2002; Bau et al. 2005), but other dynamic properties such as response duration (de Bruyne et al. 2001) and sensitivity to rate of stimulus concentration change (Tichy et al. 2005) have been noted. Response duration was linked to ORs, rather than host neurons, in Drosophila (Hallem et al. 2004).

Here, we used random binary stimulation and PID measurement to estimate frequency response functions of Drosophila basiconic antennal neurons stimulated by fruit odors. Re-
responses of single neurons fell into two distinct groups, depending on the odorant used, with opposite polarities and different frequency sensitivities. Frequency responses to mixed excitatory and inhibitory odorants were indistinguishable from those with inhibitory odorants alone.

METHODS

Animal preparation and electrophysiology

Flies, Drosophila melanogaster, Oregon R #2376 (Bloomington Drosophila Stock Center, Bloomington, IN) were raised and maintained in the laboratory using a standard diet (Lewis 1960) at a temperature of \(22 \pm 2^\circ C\) under a 13-h light/11-h dark cycle. Flies of either sex were used within 2 days of emerging. Animals were held in the cut end of a 100-µl plastic pipette tip. Tungsten electrodes were fabricated from 0.1-mm-diam wire, sharpened electrolytically by passing current through the tip into concentrated potassium hydroxide solution, and pushed into the sockets of large basiconic sensilla located near the proximal border of the posterior side of the third antennal segment. A reference tungsten electrode was inserted into the contralateral eye. Single unit recordings were fed to a Grass P55 amplifier (Grass Technologies, West Warwick, RI).

Olfactory stimulation

The stimulating system (Fig. 1) has been described previously (Schuckel and French 2008). It was constructed as a Plexiglas box (100 mm long \(\times\) 70 mm square). A 40 \(\times\) 10-mm fan (EVERCOOL EC4010M12CA, Cooler Guys, Kirkland, WA) created the primary air flow in a 120-mm-long, 13-mm-diam tube made from fluorinated ethylene propylene (Fisher Scientific, Ottawa, Ontario, Canada). The fan was driven by a 10-V DC power supply. The fly was positioned within 2–3 mm of the exit and 2–3 mm of the tube center line.

Secondary air flow came from a cylinder of compressed air containing 1,000 ppm propylene tracer gas (Linde, Halifax, Nova Scotia, Canada), regulated to 20-kPa initial pressure. It flowed through an odorant cartridge made from the shaft of a 5-ml transfer pipet (Fisher Scientific), containing a rectangular piece of filter paper (45 \(\times\) 15 mm), through a two-way (open or closed) solenoid valve (01340-02, Cole-Parmer, Montreal, Quebec, Canada) into a 16-gauge hypodermic needle with its tip located in the center of the flow tube and 42 mm from the exit. The solenoid valve used PTFE materials in contact with the gas and was driven by a 24-V DC power supply via a photovoltaic relay to be either fully open or fully closed. The valve had a specified switching time of 5 ms. Odorant chemicals and mineral oil were purchased from Sigma (Oakville, Ontario, Canada) and mixed at 20% vol/vol before final dilution. Volumes (10 µl) of each mixture were loaded into separate cartridges. Fresh cartridges were prepared for each experiment.

Propylene concentration was measured by a miniature photoionization detector (mini-PID, Model 200A, Aurora Scientific, Aurora, Ontario, Canada). The tip of the inlet probe was located directly above and within 2 mm of the fly antenna. The PID frequency response was 0–330 Hz and its concentration range was 0.05–500 ppm propylene. The PID inlet needle was 57 mm long with an ID of 0.76 mm, giving a volume of 0.0259 ml. We used a gas sampling rate of 1,150 ml/min, giving a time delay of 1.35 ms. Additional delay must have occurred as the gas passed through the ionizing chamber, so we assumed a total delay between the gas reaching the PID sample tube and the PID output signal of 2 ms.

All experiments were performed at room temperature (20 ± 2°C) and in a controlled humidity chamber (<40%). The animal preparation was mounted on an air driven anti-vibration table. The stimulating system was

![Diagram of experimental arrangement for random stimulation of Drosophila antennal sensilla](http://jn.physiology.org/doi/10.220.33.6/supplement-1/15)

**FIG. 1.** Experimental arrangement for random stimulation of Drosophila antennal sensilla. Primary air was driven by a fan through a 13-mm-diam, circular flow tube made from fluorinated ethylene propylene. Secondary air containing 1,000 ppm propylene flowed over filter paper (45 \(\times\) 15 mm) soaked in mineral oil plus dissolved odorants. Secondary air flow was switched on-off by a 2-way solenoid valve, driven by maximum-length binary sequences, and entered the primary air flow via a 16-gauge hypodermic needle. Flies were mounted in the center line of the primary air flow. Tungsten electrodes recorded action potentials from single antennal basiconic sensilla. Propylene concentration was measured by the miniature photoionization detector (PID). Traces show parts of recordings of the binary sequence, PID signal, and action potentials during an experiment using 1% ethyl acetate.
mounted separately and mechanically isolated from the preparation. The stimulation system was designed to be easily disassembled and removable from the experiment without disturbing the animal preparation. The primary air tube was changed between each different odorant to avoid wall retention, and the solenoid valve was held open with fresh air flow for a period of 5 min between experiments. Air containing odorants was removed by a fan from the experimental area via an 80-mm-diam tube connected to the building exhaust system.

**Experimental control and data processing**

All experiments were controlled by custom-written software via a personal computer and a data acquisition board (NI6035E, National Instruments, Austin, TX). Binary M-sequences to drive the solenoid valve were generated by the computer using a 34-bit binary shift register clocked at 10-ms intervals. The PID and recording electrode voltages were digitized via a 16-bit A/D converter and sampled at 0.1-ms intervals. Action potentials were detected by a template matching algorithm (Kim et al. 2004) and digitally filtered by convolution with a sinc function (French and Holden 1971) to a bandwidth of 0–50 Hz. The PID voltage (input) and filtered action potential signal (output) were resampled at 10-ms intervals.

Sampled time domain data (20,000 input-output pairs) were transferred to the frequency domain using the fast Fourier transform (Cooley and Tukey 1965) in segments of 512 sample pairs. Frequency response functions between the PID voltage and action potentials were calculated by direct spectral estimation and plotted as Bode plots of phase and log gain versus log frequency (Bendat and Piersol 1980).

Coherence, \( \gamma^2(\omega) \), as a function of frequency, \( \omega \) (Bendat and Piersol 1980), was calculated from the same data as the frequency response functions. Coherence was used to estimate the information capacity, \( R \), of olfactory transduction (Shannon and Weaver 1949)

\[
R = \int \log_2 \left[ 1 - |\gamma^2(\omega)| \right] d\omega
\]

Frequency response functions were fitted to linear filter functions (Eqs. 2 and 3) by minimizing the minimum square error between the two, each being a complex function of frequency. Error was weighted by the value of the coherence function at the same frequency.

**Results**

Experiments were performed on 84 flies, using five odorants (ethyl acetate, ethyl butyrate, phenethyl acetate, methyl salicylate, and hexanol). We attempted to test all odorants on each preparation in random order, but recordings were often lost.

![Ethyl acetate](image_url)  
**FIG. 2.** Frequency response and coherence functions between ethyl acetate concentration and action potential signal recorded from a large basiconic sensillum. Propylene concentration, measured by the PID, was assumed to be linearly proportional to odorant concentration and used as its surrogate. The total recording was of 200-s duration, and the sensilla recording contained 1 large unit and 1 much smaller unit. Large action potentials were detected by a template algorithm and digitally filtered to a bandwidth of 0–100 Hz. Estimated frequency response values (amplitude and phase shown as circles) were fitted by Eq. 2 (solid lines) giving the following parameters: \( \alpha = 3.77 \) APs/ppm, \( \tau_1 = 64.8 \) ms, \( \tau_2 = 10.6 \) ms, \( \Delta t = 1.01 \) ms. Substitution of the measured coherence function into Eq. 3 gave \( R = 18.5 \) bits/s. Inset: 1 s of the original recordings. Note that action potential firing increased with increasing odorant concentration and tended to occur on the rising phase of each increase, corresponding to the band-pass amplitude and low frequency phase advance seen in the frequency response.
before the 200 s required for a complete experiment or discarded later because of difficulty in separating action potential amplitudes or poor coherence (noisy recording). The latter criterion gave a maximum range of 10:1 for the information capacity values obtained for each odorant. Data are reported here from a total of 86 complete recordings (the similarity to the number of flies is fortuitous).

Types of sensilla recorded

Recordings from basiconic sensilla normally featured one prominent action potential, a second smaller unit (Fig. 3, inset), and sometimes a third unit. The largest unit could usually be separated from all others easily, but satisfactory separation of the second unit for the length of time required for frequency response estimation was only achieved in seven experiments. Only data from the largest unit in each recording are shown in the figures here.

Large basiconic sensilla have been classified into eight functional types, ab1–ab8 (Hallem et al. 2004), based on responses to a range of odorants. This classification also produces partial anatomical separation to different antennal regions. Four of the odorants used here were also used in the original classification (ethyl acetate, ethyl butyrate, methyl salicylate, and hexanol). The locations of our recordings and the responses to ethyl acetate and ethyl butyrate would suggest that our recordings were from types ab1, ab2, ab3, or ab7. Sensilla ab1 have four units, whereas all others have two units. Therefore the small number of recordings with more than two units were probably from ab1. However, our data for methyl salicylate are difficult to reconcile with the above classification (see DISCUSSION). Importantly, we saw no differences between

![Graph showing frequency response and coherence functions between methyl salicylate concentration and action potential signal recorded from a large basiconic sensillum. The total recording was of 200-s duration, and the sensilla recording contained 1 large unit and 1 small unit. Estimated frequency response values (amplitude and phase shown as dots) were fitted by Eq. 3 (solid lines) giving the following parameters: \( \alpha = -0.269 \) AP/s/ppm, \( \tau_i = 35.7 \) ms, \( \tau_d = 3.23 \) ms, \( \Delta t = -0.88 \) ms. Substitution of the measured coherence function into Eq. 1 gave \( R = 7.9 \) bits/s. Inset: 1 s of the original recording. Note that action potential firing increased with decreasing odorant concentration and tended to occur once a low concentration was established, corresponding to the low-pass amplitude and phase of the frequency response.]
the dynamic properties of different recordings that could be based on the types of sensilla recorded.

**Excitatory and inhibitory responses**

Frequency response functions were measured between propylene concentration (input signal) and action potentials (output signal), with propylene acting as a surrogate for odorant concentration. Control experiments without odorants were performed on animals immediately before adding odorant cartridges. These experiments failed to show any detectable response of single units to propylene, similarly to previous control measurements from *Drosophila* electroantennograms receiving propylene alone (French and Meisner 2007). We showed previously that the frequency response of the stimulation system is low-pass with a corner frequency of \( \approx 35 \) Hz (Schuckel et al. 2008), so the stimulus was approximately white noise over the animal’s response range, although whiteness is not essential for frequency response measurement by spectral estimation.

Frequency response functions fell into two clearly separable groups, based on their overall form, and particularly on the phase relationship. In 57% of recordings, the phase relationship indicated that increasing odorant concentration caused increased action potential firing, or excitation, but in the remaining 43% of recordings, the phase value was \( \sim 180^\circ \) at low frequencies, representing an inverted, or inhibitory, response.

Excitatory responses were always seen with stimulation by ethyl acetate, ethyl butyrate, or hexanol. Their frequency response functions (Fig. 2) could be well-fitted by the band-pass relationship

### Figure 4

Summary of fitted parameters to Eqs. 1–3 for all experiments. All data are shown as mean ± SE values, and the numbers of experiments are shown below. Ethyl acetate was loaded onto the filter paper at 1% in mineral oil. All other odorants were loaded at 10%. Negative values of the amplitude parameter, \( \alpha \), correspond to an inverted response, as seen in Fig. 3.
Responses to mixed excitatory and inhibitory odorants

In three experiments, we recorded frequency responses of the same unit to ethyl butyrate alone (excitatory response), phenylethyl acetate alone (inhibitory response), and an equal mixture of the two odorants. The odorants were mixed before loading to give the same concentration of each odorant as was presented individually. In each case, the response to the mixture was clearly inhibitory and the frequency response function was low-pass (Fig. 5). The amplitude values obtained from the mixtures (mean $\alpha = -0.64$ APs/ppm) were close to those from methyl salicylate alone (mean $\alpha = -0.59$ APs/ppm), even though ethyl butyrate alone gave a mean positive amplitude of $\alpha = 3.87$ APs/ppm, indicating that the inhibitory odorant completely suppressed the response to the excitatory odorant.
the entire range. For excitatory odorants, there was a clear tendency to reach peak firing rates earlier as concentration rose, reflecting the phase advance seen at low frequencies in the frequency response functions (Fig. 2).

Responses of smaller units

In a total of seven cases, we were able to separate smaller action potentials sufficiently well to estimate frequency response functions (2 ethyl acetate, 2 phenylethyl acetate, 3 methyl salicylate). Six of these showed low-pass inhibitory responses. The seventh, with methyl salicylate, gave a low-pass excitatory response.

DISCUSSION

Responses of Drosophila basiconic sensilla neurons to fruit odorants were clearly divided into two groups based on both their polarity and dynamics. Excitatory responses were seen with ethyl acetate, ethyl butyrate, and hexanol, which are all aliphatic compounds, whereas inhibitory responses were seen with phenylethyl acetate and methyl salicylate, which are both aromatic compounds. Responses to equal mixtures of excitatory and inhibitory odorants could not be distinguished from responses to inhibitory odorants alone.

Physiological basis of the dynamic parameters

Interpretation of the amplitude parameter, $\alpha$, is impossible at this time because we do not know the ratios of different odorant concentrations to the propylene surrogate. Although the mean firing rates were higher for excitatory odorants, and the sensitivities to excitatory odorants were all $\sim10$ times greater than to inhibitory odorants (Fig. 5), this could reflect relative vapor pressures rather than any physiological mechanisms. However, the dose-response estimates (Fig. 6) suggested that the concentrations used were within the linear operational ranges of the receptors for these odorants and were compatible with dose response measurements for several Drosophila odorants that gave linear response ranges over more than two log units of concentration (Pelz et al. 2006).

Excitatory olfactory responses had band-pass frequency responses, typically reaching peak values in the range of 1–10 Hz (Fig. 2). Correspondingly, they showed a phase lead at low frequencies, changing to a lag at high frequencies. Excitatory responses could be fitted by the band-pass filter of Eq. 2, with long and short time constants corresponding to the rising response at low frequencies and the falling response at high frequencies, respectively. These dynamic properties were similar for all excitatory odorants tested. In contrast, inhibitory responses had low-pass frequency responses, with constant
asymptotic amplitude at low frequency and matching phase responses (Fig. 3). These responses were fitted well by the second-order filter of Eq. 3. Again, these dynamic properties were similar for both inhibitory odorants tested.

The decreasing response at high frequencies seen with all odorants was also reported for electroantennogram responses of moth antennae to pheromones (Justus et al. 2005) and Drosophila antennae to fruit odorants (Schuckel et al. 2008). Time-dependent steps responsible for reduced response to rapidly changing inputs could include odorant diffusion through cuticular pores, binding to extracellular proteins in the lymph space that surrounds the neurons, binding to membrane odorant receptor molecules or intracellular second messenger cascades (Benton 2007; Benton 2008). The requirement for two time constants in the inhibitory responses suggests that more than one mechanism may be involved in this feature of the responses.

Stimulation with a constant concentration of odorant would correspond to zero frequency, so the decreased response at low frequencies seen with excitatory odorants can be viewed as adaptation to a maintained stimulus. Decreased sensitivity to low frequencies, or adaptation, is widely observed in other sensory systems, such as mechanoreception, and several extracellular and intracellular mechanisms can contribute to it (French 1992). Lower sensitivity to slow changes in concentration has also been reported in cockroach olfactory receptors (Tichy et al. 2005). Possible mechanisms for adaptation in Drosophila olfactory neurons could include time-dependent gating of odorant receptors, intracellular signaling, or adaptation of action potential encoding. Previous frequency response measurements of Drosophila antennograms failed to show significant adaptation with time after initial presentation of fruit odorants (Schuckel et al. 2008), but it is impossible to measure frequency responses within the adaptation times of ~100 ms that are typically seen with pulsed odor presentations (de Bruyn et al. 2001).

The additional time delay, $\Delta t$, required to fit the phase relationship was relatively small. Detection of propylene by the PID contributed about ~2 ms to this parameter, eliminating any significant delay for ethyl butyrate or phenylethyl acetate. Including this correction gave values of ~7 ms for ethyl acetate and 9 ms for methyl salicylate. It is possible that these have a physiological basis, such as passage through cuticular pores. This may be clarified in the future when data becomes available for a broader range of odorants.

The coherence function, $\gamma^2(\omega)$, was comparatively high (its maximum possible value is 1), but the limited frequency range of the responses reduced the estimated information capacity, $R$, to a maximum of ~10 bits/s, which is much lower than comparable measurements in mechanoreceptors or some photoreceptors of $\approx 200$ bits/s (Juusola and French 1997). Information capacity varied with different odorants but was approximately proportional to response amplitude, $a$. Because information capacity in a linear system measures signal-to-noise ratio, this indicates that the background noise level in the neurons was relatively independent of the odorant.

The coherence function can be lowered by noise or by nonlinearity. The lower values at low frequencies, particularly

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**FIG. 7.** Some hypothetical models of interacting excitatory and inhibitory odorants in a single olfactory neuron. A: odorant receptor molecules could have multiple binding sites with opposite effects on the cation channel. This would require a background level of channel opening that could be increased or decreased. B: separate odorant receptor molecules would allow different mechanisms to be used for excitatory and inhibitory odorants but would also require background excitation. C: potassium or chloride channels in neuronal membrane proximal to the sensory epithelium could hyperpolarize the neuron. This would not require a background level of excitation. D: both excitatory and inhibitory effects could occur within a single odorant receptor molecule if it functions as an ion channel with a suitable set of closed states.
for excitatory compounds, could reflect dependence of sensitivity on concentration or other nonlinearities. Justus et al. (2005) found significant nonlinear compression of moth antennal responses to pheromone using similar techniques. Although detailed nonlinear analysis is beyond this description, future work should address the issue of response linearity, using nonlinear systems analysis over a wider range of odorant concentrations.

Differences to previous data

Methyl salicylate has previously been characterized as excitatory or inactive for all Drosophila basiconic sensilla neurons (de Bruyne et al. 2001) and ORs (Hallem et al. 2004), whereas we only observed clearly inhibitory responses. However, only neuron ab1D, the smallest unit of the sensilla containing four neurons, has previously been identified as strongly excited by methyl salicylate (de Bruyne et al. 2001; Hallem et al. 2004). We did not characterize smaller units well enough to test this, but it might explain the single excitatory response to methyl salicylate from a smaller unit that we observed. It is possible that the inhibitory effects of methyl salicylate on large units were not observed previously because of the pulsatile testing method used.

Our excitatory and inhibitory responses were obtained with aliphatic and aromatic compounds, respectively, but previous data has shown all four possible combinations of excitatory and inhibitory responses to aliphatic and aromatic compounds (de Bruyne et al. 2001; Hallem et al. 2004; Shields and Hildebrand 2001).

Mechanisms of excitatory and inhibitory responses in single neurons

Current theories of Drosophila olfaction suggest that individual members of the family of odorant receptor molecules (ORs) can each combine with one member of the family (ORR83b) to form dimeric odor-activated cation channels whose odor specificity is controlled by ORx (Benton 2008; Sato et al. 2008; Wicher et al. 2008). Our data show that individual olfactory neurons can respond with opposite polarity and different dynamics to different odorants and that excitatory and inhibitory odorants can interact sufficiently strongly that a normal excitatory response can be completely suppressed by an inhibitory odorant. A range of possible transduction schemes could account for these findings (Fig. 7).

1) Excitatory and inhibitory sites could be on the same receptor molecule, with the inhibitory odorant preventing response to excitatory odorants (Fig. 7A). This would require a background level of cation channel opening that inhibitory odorants could inhibit and allow rebound excitation as they were removed. Supporting this, olfactory neurons usually display background firing in the absence of odorants. The differential dynamics of the two processes would have to be caused by time-dependent processes within the receptor molecules. A model with some similarities was proposed previously with a single binding site (Hallem et al. 2004).

2) A related scheme would have separate receptor molecules for excitatory and inhibitory odorants converge on the same cation channel (Fig. 7B). This would also require background channel opening. It is more difficult to reconcile with the idea of direct, receptor-activated cation channels, but might allow easier separation of the dynamics. Hallem et al. (2004) expressed excitatory and inhibitory ORs in a single neuron, and found intermediate responses to a range of odorants, whereas our data always showed dominance by one response, arguing against this possibility in normal neurons.

3) Olfactory receptor neurons are located in a sensory epithelium that isolates their distal dendrites within a lymph space. However, more proximal regions of the cell are exposed to interstitial fluid beneath the epithelium, so potassium channels in this region could hyperpolarize the neuron (Fig. 7C). This would not require background excitation and would explain the rebound excitation, but would require a second messenger system, which seems less likely, given the similar high-frequency dynamics of excitatory and inhibitory signaling.

4) A single receptor molecule cation channel could mediate excitation and inhibition via multiple closed states (Fig. 7D). Inhibitory odorants could direct the molecule into a second closed state that is refractive to excitatory odorants, so that removal of the inhibitory odorant would allow relaxation to a normal closed state or through an open conformation, as shown here. This model would not require background excitation and could show rebound excitation (Fig. 3). In this case, the different dynamics seen in excitation and inhibition would involve the molecular conformations responsible for the state changes. However, the falling sensitivity at high frequencies is common to both functions and could arise from other transduction steps, as discussed above.

Characterization of the dynamic properties of olfactory neurons not only provides a direct measure of excitatory versus inhibitory behavior but promises to offer important new insights into olfactory mechanisms and behavior. Inhibition is probably a more widespread property of olfactory receptors than previously suspected, and interactions between odorants at the receptor level may play important roles in olfactory sensation. Exploration of dynamic information transmission beyond the primary neurons to higher centers, as well as the use of a much broader set of odorants should now be possible.

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REFERENCES


