Dynamic properties of antennal responses to pheromone in two moth species

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Abstract

Dynamic properties of pheromone plumes are behaviorally important in some moths for inducing upwind flight, but little is known about the time dependent properties of odor transduction or the mechanisms that limit receptor dynamic sensitivity. We stimulated male antennae of two moth species, *Cadra cautella* and *Spodoptera exigua*, with pheromone plumes in a wind tunnel while recording electroantennograms (EAG) and concentration of a surrogate plume (propylene, which mimics a pheromone plume) using a photoionization detector (PID). Turbulent plumes were produced by mechanical baffles, creating broad frequency range dynamic concentration changes at the antennae. Frequency response functions and coherence functions between PID and EAG signals were used to measure the dynamic responses of the two species to pheromone blends and individual components. A single time constant filter fitted the responses of both species, but *S. exigua* was about three times faster than *C. cautella*. Responses to individual pheromone components were significantly different in *S. exigua* but not in *C. cautella*. We also fitted the data with a simple block-structured nonlinear cascade. This supported the simple filter model but also suggested that the response saturates at an early stage of chemotransduction.
INTRODUCTION

Moth antennae have numerous sensilla for the detection of air-borne odorants, including host plant odorants and sex pheromones. Sensillar morphologies are complex, but antennal sensilla of male moths that detect female pheromones usually contain 1-3 sensory neurons, with each neuron being highly sensitive and specific to a single pheromone component. In species having multi-component pheromones, specific neurons for each component are housed either in the same sensillum (e.g., *Yponomeuta* spp., Van Der Pers and Den Otter 1978) or in separate sensilla (e.g., heliothine moths, Cossé et al. 1998; Baker et al. 2004). Chemotransduction mechanisms in arthropod sensory neurons are complex, with several second messenger pathways and ion channels involved (Ache et al. 1998), so that interactions between different odorant molecules may be processed at the single sensory cell level.

In addition to molecular specificity, olfactory systems may detect dynamic properties of odorant signals as their concentrations change with time. Natural odor plumes are intermittent because variable wind direction and velocity shreds the plume into discrete filaments of varying concentration that are interspersed with odor-free air (Murlis et al. 1992). Intermittent arrival of fluctuating concentration is important for male moths orienting along a pheromone plume because it may overcome densensitization and/or adaptation of sensory processes (Willis and Baker 1984). Plume temporal structure may also provide information about the distance to the odorant’s source (Murlis et al. 1992; Justus et al. 2002a).

Behavioral evidence shows that the trajectory and velocity of a moth flight track is determined by this fine-scale structure of the odor plume (Willis and Baker 1984; Mafra-Neto and Cardé 1995; Justus et al. 2002b). Navigation along a pheromone plume occurs when a male moth
intercepts a filament, causing a brief upwind surge. In the absence of a second encounter with pheromone, the moth returns to cross wind-directed flight (Mafra-Neto and Cardé 1998), but given an optimal temporal frequency of filament detection, it can orient along the plume to its source in a relatively straight trajectory if wind direction is approximately constant (Mafra-Neto and Cardé 1998; Justus et al. 2002b).

Dynamic structures of pheromone plumes provide important information to male moths, and neural substrates for decoding such information have been proposed in the central nervous system (Vickers et al. 2001), but little is known about the dynamic responses of the antennal sensory neurons that detect the odorant signal. Electroantennogram (EAG) experiments with pulsed pheromone plumes in a laminar flow wind tunnel suggested that regularly pulsed stimuli at frequencies up to 30 Hz or more can be resolved by some moth species (Bau et al. 2002). However, as described above, pheromone rarely arrives at an antenna as a regularly pulsed signal, but rather as an irregular signal with a complex temporal spectrum.

In an effort to understand the time dependence of pheromone-sensitive neurons in male moths, we measured the dynamic sensitivities of *Cadra cautella* and *Spodoptera exigua* antennae by stimulating them with turbulent pheromone plumes that mimic natural complexity and provide a broad frequency range of concentration changes suitable for direct spectral analysis. Frequency response functions between pheromone concentration input, measured by a surrogate odor, and electroantennogram output were well-fitted by single time constant filter functions that passed low frequencies but progressively reduced high frequencies. Fitted parameters from spectral analysis were used to compare responses from different species and between different components of pheromone blends. Nonlinear block-structured dynamic simulations also supported the low-pass
filter model, but additionally suggested that response saturation occurs early in the odorant transduction mechanism.
MATERIALS AND METHODS

Insects

_Cadra cautella_ were reared on a diet of poultry laying mash, rolled oats, brewer’s yeast and glycerin (Mafra-Neto and Cardé 1995). Last instar larvae were sexed and males were isolated in plastic cups (177 ml, 7 cm diameter base) until emergence. _Spodoptera exigua_ were reared on a lima bean diet (modified from Shorey and Hale 1965). Males were sexed as pupae and placed in plastic cups (see above) until emergence.

Pheromone

Synthetic pheromone solutions were prepared gravimetrically in hexane (10 μg/μl) and serially diluted to obtain pheromone blend and single component concentrations of 10 ng/μl. Pheromone blends consisted of a 10:1 mixture of (Z,E)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac) and (Z)-9-tetradecenyl acetate (Z9-14:Ac) (purities of 93.9 and 96.3% respectively; Bedoukian Research, Danbury, CT, USA) for _C. cautella_, and a 10:1 mixture of Z9,E12-14:Ac and (Z)-9-tetradecenol (Z9-14:OH) (purities of 93.9% and 93.6% respectively; Bedoukian) for _S. exigua_.

We use the terms ‘major’ and ‘minor’ to refer to the quantity of each component in the blend. They do not necessarily reflect the quantities produced by a female, which are highly variable in both _C. cautella_ (J.D. Allison, personal communication) and _S. exigua_ (Persoons et al. 1981).

Wind tunnel and odor delivery

All recordings were carried out in a three meter long, push-pull wind tunnel consisting of a Plexiglas® floor and Lexan® sheeting bent into semi-cylindrical shape with a center height of 1.5 m
(see Justus et al., 2002a). Laminar flow was achieved by way of a 15 cm thick aluminum honeycomb (Hexel® 15 mm diameter cells) at the tunnel’s upwind entrance. Wind speed was set at 50 cm/s via variable speed motors operating the upwind and downwind fans, and verified using a digital anemometer. The inner surface of the tunnel was covered in aluminum foil, to shield it from electrical interference. The tunnel was housed in a temperature and humidity controlled room, at 25°C and ~65% R.H.

A pressurized cylinder of a tracer gas (propylene, 1000 ppm) was connected via polypropylene tubing to a Stimulus Flow Controller (SFC-2, Syntech, Hilversum, The Netherlands). The tracer gas was introduced into the tunnel at a rate of ~3.0 ml/s via a Pasteur pipet that was bent at 135° angle with the aperture of the pipet facing upwind. To entrain pheromone into the flow, a 7.5 mm filter paper disk impregnated with 20 ng/μl pheromone was inserted into the Pasteur pipet and positioned near the outlet of the pipet (to minimize adsorption to the inner surface of the pipet).

Artificial turbulence within the wind tunnel was created by attaching a baffle to the downwind side of the pipet. The baffle consisted of a circular paper disk that was either 22 mm (small baffle) or 55 mm (large baffle) in diameter (Fig. 1). Two baffles were used to test the effects of baffle size on the temporal spectrum of the odor plume. Pipets and baffles were replaced with freshly prepared setups for each antennal preparation.

*Photoionization detector (PID) and electroantennograms (EAG)*

Male moths (2 days post-eclosion) were chilled in the refrigerator for 2 minutes. The left antenna was excised at the pedicel, using a razor blade coated with electrode gel (Spectra 360, Parker Laboratories Inc., NJ, USA), and mounted between recording and reference electrodes using
electrode gel (Fig. 1). The antennal preparation was then set into the EAG probe (PRG-2, Syntech), perpendicular to the airflow with the ventral side facing upwind (Fig. 1). The stand holding the EAG probe was electrically grounded to the aluminum foil lining the tunnel to inhibit interference. The probe was situated within the tunnel so that the antenna was positioned 25 cm above the floor, and 25 cm downwind of the circular baffle at the approximate center of the propylene-pheromone plume.

A miniature photoionization detector (miniPID; Aurora Scientific, Aurora, ON, Canada) with a frequency response of 330 Hz and a detection threshold of 50 ppb was set into the wind tunnel at 25 cm above the floor, with the inlet of the miniPID inline with the center of the circular baffle, and directly behind (~1.0 mm) the antennal preparation (Fig. 1).

Each antenna was first tested to ensure that there was no mechanical response to the air flow in the absence of pheromone. Then, each was recorded for a total of eight minutes: four minutes with each baffle and two minutes between recordings. Small and large baffles were presented in random order. Signals were acquired through a PC interface board (IDAC-02, Syntech) using Autospike32 (Syntech) software. PID signals were filtered above 3 kHz. EAG signals were amplified (x10) and filtered below 0.1 Hz and above 3 kHz.

Frequency domain measurements

EAG and PID signals were sampled at regular intervals of 1.05 ms giving a Nyquist frequency of 476 Hz (Bendat and Piersol 1980), but preliminary experiments showed that neither signal contained significant power at frequencies higher than 50 Hz, so all data were re-sampled by averaging adjacent points to give an effective interval of 5.25 ms. Sampled signals were transferred to the frequency domain using the fast Fourier transform (Cooley and Tukey 1965) in segments of
1024 sample pairs, giving 44 ensembles. Frequency response functions (gain and phase) between the PID and EAG were calculated by direct spectral estimation and plotted as Bode plots of phase and log gain versus log frequency. Frequency response functions, $G(f)$, were fitted by a coherence-weighted minimum square error procedure to a first-order low-pass filter function:

$$G(f) = \frac{\alpha}{(1 + j2\pi f \tau)}$$

(1)

where $f$ is frequency, $\alpha$ is a constant amplitude parameter, $\tau$ is the filter time constant and $j = \sqrt{-1}$. Since the phase lag progressively exceeded the value predicted from the gain with increasing frequency, a time delay, $\Delta t$ was added to the fitting function. The combined low-pass filter and time delay were fitted to the complex frequency response function before separation into gain and phase, giving three fitted parameters, $\alpha$, $\tau$ and $\Delta t$.

The coherence function, $\gamma^2(f)$ was also calculated from the direct spectral estimation (Bendat and Piersol 1980). Coherence is a normalized correlation function, where a value of unity indicates linear, noise-free signal transmission, whereas values less than unity indicate nonlinear signal transformation or added noise that is not correlated to the input or output signals.

For a linear system the signal-to-noise ratio, $SNR$, is related to coherence by:

$$SNR(f) = \frac{1}{1 - \gamma^2(f)} - 1$$

(2)

(Bendat and Piersol 1980), and the total information capacity of the system as a communication channel, $R$, can be obtained from $SNR$ (Shannon and Weaver 1949) by:
\[ R = \int \log_2(1 + \text{SNR}(f))df \]  

(3).

From equations (2) and (3), coherence was used to estimate the information capacity of odor detection via:

\[ R = \int_0^\infty \log_2\left(\frac{1}{1 - \gamma^2(f)}\right)df \]  

(4)

**Nonlinear time domain measurements**

Data were also fitted by a nonlinear block structured model (Hunter and Korenberg 1986; French and Marmarelis 1999) consisting of a static nonlinearity followed by a low-pass linear filter (NL model, see Fig. 5). The static nonlinearity was a third-order polynomial function:

\[ w(t) = b_0 + b_1x(t) + b_2x^2(t) + b_3x^3(t) \]  

(5),

where \( x(t) \) was the input (PID signal in this case) as a function of time, \( t \), and \( b_j \) were the polynomial coefficients to be fitted. The linear filter had impulse response:

\[ g(u) = \frac{1}{\tau}e^{-u/\tau} \]  

(6)

where \( u \) was a time variable and \( \tau \) was the time constant to be fitted, as in equation (1).

Fitting of the NL model to the data was performed on the first 40,000 input-output data pairs by simulated annealing (Press et al. 1990) to minimize the mean square error (MSE) between EAG output, \( y(t) \), and the simulated output, \( y_s(t) \):
\[ MSE = 100 \cdot \frac{(y(t) - y_s(t))^2}{y^2(t) - (\bar{y}(t))^2} \]

where the bars indicate time averages (French and Marmarelis 1999).

Data processing

All data processing was performed on IBM-compatible personal computers using custom-written software. Statistical analysis was performed with Prophet 6.0 software (AbTech Corporation, Charlottesville, VA, USA). Differences between responses of different species and different pheromone components were tested using a two-sided unpaired t-test.
RESULTS

Antennal responses can be approximated by first-order low-pass filter functions

Two sizes of baffles were tested in the wind tunnel for their ability to create turbulent pheromone plumes that would deliver a wide range of temporal frequency components to the antennae, as measured by the photoionization detector (PID). Both baffles gave detectable signal power in the range from 0-30 Hz, with the larger baffle giving relatively more power at lower frequencies (Fig. 1). However, no systematic differences were seen between frequency response functions or coherence functions obtained with the two baffle sizes. Therefore, results from experiments using the two configurations were combined for all further analysis. Segments of typical raw PID and EAG recordings are shown in Figure 5.

The gain portions of frequency response functions between pheromone concentration (PID) as input, $x(t)$, and electroantennogram (EAG) as output, $y(t)$, always had low-pass form, and were well-fitted by a simple low-pass filter function with a single time constant (Equation 1). However, experimental phase data always lagged progressively behind the filter function at higher frequencies, indicating the presence of an additional time delay. The fitted curves in Figure 2 include this additional time delay. Coherence functions were relatively high, often approaching unity over the range 1-10 Hz, indicating that EAG gives an approximately linear, noise-free representation of odorant concentration in this frequency range.

Coherence was always reduced at frequencies below about 1 Hz. This reduction was often accompanied by reduced gain at the lowest measured frequencies. The most likely cause for these effects is adaptation of the receptor cells, causing a reduction in gain at low frequencies and a consequent reduction of signal-to-noise ratio, leading to reduced coherence. Adaptation of action
potential discharge to low frequency stimuli is common in sensory receptors, and is most often seen as a slowing or cessation of response to a constant stimulus, such as the time after a step change in displacement of a mechanoreceptor (French and Torkkeli 2001). Adaptation to low frequencies has been observed previously in single moth pheromone receptors (Carlsson and Hansson 2002).

Coherence was also reduced at frequencies above about 10 Hz in both species. Part of this reduction must have been due to the decreasing gain of the receptors. However, the different sensitivities and time constants of the two species were not strongly reflected in the coherence reductions (Fig. 2), indicating that experimental loss of input power at high frequencies (Fig. 1) was the major cause of reduced coherence.

The coherence function was integrated over frequency to give an estimate of information capacity (Equation 4) for each experiment. Note that this estimate is based on an assumption of linearity (Equations 2 and 3), which is probably not strictly true in this case.

S. exigua and C. cautella have different dynamic responses to pheromones

The fitted first-order low-pass filter function (Equation 1) has two parameters: amplitude, $\alpha$, and time constant, $\tau$, corresponding to the overall sensitivity of olfaction and the ability to discriminate changing concentrations of pheromone with time, respectively. Spodoptera exigua showed increased sensitivity (higher $\alpha$) and better temporal discrimination (lower $\tau$) than C. cautella (Fig. 2) and these differences were seen in all the experiments (Fig. 3), with S. exigua being about twice as sensitive ($\alpha=1.48\pm0.12$ versus $\alpha=0.64\pm0.14$, mean $\pm$ standard error) and more than three times faster ($\tau=15.1\pm1.6$ ms versus $\tau=50.2\pm3.9$ ms) on average. However, no significant differences were seen in the time delay ($\Delta t=5.72\pm0.67$ ms versus $\Delta t=5.71\pm0.63$ ms) or information capacity
(R=24.8±0.8 Bits/s versus R=23.5±1.3 Bits/s) between the two species under these conditions.

S. exigua dynamic response varies with pheromone components

Fitted filter parameters, time delays and information capacities were also measured during stimulation with each of the major and minor components of the pheromone blends for the two moth species (Fig. 4). No significant differences were seen between any of the parameters obtained using the major or minor components of C. cautella (α=0.92±0.01, α=0.73±0.09, τ=76.0±8.2 ms, τ=74.2±6.8 ms, Δt=7.22±1.60 ms, Δτ=8.31±1.15 ms, R=18.1±0.7 Bits/s, R=18.7±1.8 Bits/s, major versus minor). However, for S. exigua there were significant differences in all of the parameters (α=0.73±0.10, α=0.41±0.07, τ=15.9±1.8 ms, τ=24.6±3.2 ms, Δt=0.60±0.52 ms, Δτ=7.61±0.91 ms, R=36.9±1.9 Bits/s, R=17.3±2.3 Bits/s, major versus minor). The major component produced larger and faster responses with smaller delays. Information capacity was also higher during stimulation with the major component, indicating that it produced a higher signal-to-noise ratio.

Nonlinear properties of pheromone responses

Coherence values below unity can indicate noise or nonlinearity in a system. To test for nonlinear behavior we used block-structured nonlinear cascade models (NLN models) based on static nonlinear and dynamic linear components (Hunter and Korenberg 1986; French and Marmarelis 1999). NLN models were fitted to the original input-output time domain data using the criterion of minimum mean square error (MSE, Equation 7). In a preliminary analysis we tested models with NL, LN and NLN structures. The linear (L) component was always a first-order low-pass filter with exponential impulse function, as predicted from the frequency response results
The nonlinear (N) components were polynomial functions. We used the criteria that adding an additional polynomial term must reduce MSE by at least 1%, but always tested an additional term when this criterion failed, to allow fitting of strongly odd or even nonlinearities. The mean delay obtained from frequency response functions was about 5.7 ms (Fig. 3), corresponding to about one sample interval in the time domain (5.25 ms). Therefore, we tested all models with fixed delays between input and output of zero, one and two sample intervals. A delay of one sample interval consistently gave the lowest MSE values, and was used throughout.

The most satisfactory results were obtained with an NL model having a third-order polynomial component (Fig. 5). Fitted values of the linear filter time constant agreed closely with those obtained from frequency response functions (Fig. 6), but the fitted linear slope of 1.13 suggested that frequency response measurements slightly underestimated the time constant. The time constant results for small and large baffles are also separated in Figure 6 to illustrate the lack of any effect due to baffle size.

Model predictions followed the experimental results but often failed to match extreme fluctuations (Fig. 5). This was probably due to noise rather than additional nonlinearity, because model predictions often matched or exceeded large fluctuations. Experimental records also showed high frequency noise that was not reproduced by the models (Fig. 5). The static nonlinear components always showed mild compression, or saturation with increasing stimulus input (Fig. 6). This was more pronounced in *S. exigua* than *C. cautella*. The initial slopes of these relationships were similar to the amplitude values obtained from frequency response functions (Fig. 3).
DISCUSSION

Antennal resolution of pheromone plume dynamics

Our initial objective in conducting these experiments was to quantify the dynamic responses of moth antennae to pheromone plumes. The high coherence of the frequency domain measurements, and the close agreement between the time and frequency domain parameter estimates, indicate that a first-order low-pass filter model provides a good estimate of electroantennogram (EAG) responses. The filter function enables us to predict the amplitude of EAG response at any frequency, and would allow us to place quantitative limits on pheromone plume detection if we knew the threshold sensitivity of the next stage in the olfaction system. For example, we can say that EAG amplitude is reduced by a factor of ten at about 32 Hz in *C. cautella* and about 106 Hz in *S. exigua*. These estimates agree with previous EAG measurements using regularly pulsed stimuli in a laminar flow wind tunnel in which both species could resolve pheromone pulses up to at least 33 Hz, with *S. exigua* giving more robust responses at high frequencies (Bau et al. 2002).

Physiological basis of EAG dynamic responses

EAG signals are produced by receptor and/or action potentials propagating along the antennal nerve from sensory receptor cells. Which stages of olfaction could be responsible for the dynamic properties of the EAG that we observed? The low-pass filter time constants were significantly longer than most neural membrane time constants, particularly in the case of *C. cautella*, so that conversion of receptor current to receptor potential should not be involved. Action potential encoding is usually associated with high-pass behavior due to sensory adaptation (French and Torkkeli 2001) and adaptation of action potential firing has been observed in single moth
pheromone receptors (Carlsson and Hansson 2002) so that the low-pass filtering probably arises before encoding. Therefore, the low-pass stage probably occurs between pheromone arrival at the sensilla and opening of ion channels in the sensory neuron membrane due to chemotransduction. Processes such as pheromone diffusion through cuticular pores, binding to extracellular proteins, binding to membrane receptor molecules, or second-messenger cascades could all contribute such time-dependent properties.

The nonlinear component of the NL cascade model (Fig. 6) indicated that receptor responses saturated as pheromone concentration (measured by PID) increased in the range that we used, particularly for S. exigua. The NL model further suggested that saturation occurs before the low-pass filter. This could be explained by processes such as saturation of odorant pores, or odorant binding proteins, or saturation of receptor molecules on the sensory cell membrane, if the low-pass filter is due to an intracellular second messenger cascade.

The location of the PID system 1 mm downwind of the antenna must have created an artifactual time delay in the opposite direction to our observed time delays. A steady wind speed of 50 cm/s would cause the antenna to receive the pheromone 2 ms before the PID. This value must have varied because of the turbulent structure of the plume, but on average our experimental delay measurements should be increased by about 2 ms.

Time delays in neural systems are often caused by action potential propagation. Little quantitative information is available about conduction velocities in arthropod nerve axons. Chapman and Pankhurst (1967) measured conduction velocities in a range of cockroach sensory axons, and found an approximately square root relationship between conduction velocity and axon diameter. For example, their data predict that axons with a diameter of about 1 μm, have a conduction velocity
of about 1 m/s. We measured the filiform antennae of *C. cautella* and *S. exigua* to be approximately 4.5 mm and 7 mm long, respectively. Therefore, the delays of 5-10 ms that we observed could be explained by conduction along the antennae, given a reasonable range of axon diameters.

Our data also showed that EAG responses in *S. exigua* are about three times larger than in *C. cautella*. This could reflect more sensory axons firing, or larger diameter axons in *S. exigua*. Axonal diameters have previously been correlated with receptor responses (Kaissling and Thorson 1980). For example, the acetate-sensitive cell of *Antherea polyphemus* is larger than the aldehyde-sensitive cell and produces larger action potentials.

The lineages of these two moth species are long-diverged, and one would expect there to be moderate differences in their peripheral senses, including their abilities to resolve rapidly flickering signals of the kind in our turbulent plumes. In addition, these two species have very different habitats in nature, with very different wind conditions that alter the structure of odor plumes. *Cadra cautella* is a pest of stored products and is typically found in enclosed areas with little or no wind, whereas *S. exigua* is a pest of field crops and would naturally experience predominantly windy conditions.

**Responses to individual pheromone components**

Frequency domain analysis of EAG responses to the major and minor pheromone components produced no detectable differences in any of the measured parameters for *C. cautella*, and the response amplitudes for the major and minor components alone were not additive. The most parsimonious explanation for this result in isolation would be that a single type of receptor cell is responsible for detecting both components. Alternatively, if two different neurons are used to detect
the two components, they must have similar sensitivities, time constants and conduction velocities. Male *C. cautella* perform pheromone-specific behaviors (e.g., wing fanning and oriented flight) in the presence of female pheromone. A blend of synthetic Z9-E12:Ac and Z9:Ac in a 10:1 ratio produces those same behaviors, as does the major component (Z9-E12:Ac) alone, although their characteristics are different from those with the blend. However, the minor component alone provokes no behavioral response from the male (J.D. Allison, personal communication), making it unlikely that a single receptor cell type detects both components. If the two components are detected by two cells occupying a single sensillum, as in *Yponomeuta* spp. (Van Der Pers and Den Otter 1978), our results may reflect some interaction between the two cells that limits the maximum response from the sensillum.

In contrast, the EAG responses of *S. exigua* to its major and minor pheromone components were different in all measured parameters. The simplest explanation for these results is that separate receptor neurons exist for each of the two components, with the receptor cell for the major component having a shorter time constant. The reduced time delay and larger response amplitude could both be explained by these neurons having larger diameter sensory axons. The increased information capacity for the major component indicates a higher signal-to-noise ratio, which is probably due to a larger response amplitude.

*Signal transmission by EAG*

The information capacity values recorded here are significantly lower than typical values of more than 200 Bits/s obtained in other spiking sensory neurons such as spider mechanoreceptors, and much lower than the values of more than 2000 Bits/s that are common in non-spiking neurons
(Juusola and French 1997). More comparable values have been observed in insect photoreceptors at low light levels (Niven et al. 2003). The low information capacity reflects the relatively high noise level visible in the original recording (Fig. 5), but it is not yet possible to tell if the noise is mainly due to irregularity in the receptor cell firing itself, or action potentials from other neurons than the primary chemoreceptor cells. Mechanosensory neurons of antennae are well-known in insects, including Lepidoptera (see Schneider 1964, Zacharuk 1985). However, antennal responses to wind alone in the tunnel did not produce electrical noise above the baseline signal in EAGs.

**Conclusions**

Our results show that we can deliver defined, broad-band stimuli to primary olfactory neurons, allowing accurate estimation of their dynamic properties in the time and frequency domains, and providing a pheromone signal that approximates dynamic natural stimuli. The resulting EAG responses provide new information about the dynamic ranges of different species and some discrimination between the properties of receptor neurons for different pheromone components. In future, responses of individual receptor neurons to similar broad-band stimuli should provide more detailed information about the dynamics of olfactory transduction. Understanding the time dependence of chemoreceptor cells will be an essential step in creating complete models of animal behavior when odor is the major sensory signal.
Acknowledgments

We thank Mr. Tedros Berhane for rearing colonies of *C. cautella* and *S. exigua*. We also thank Päivi Torkkeli and Jeremy Allison for critical reading of the manuscript.

Grants

This work was supported by a grant from the Canadian Institutes of Health Research to A.S.F.
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FIGURE LEGENDS

FIG. 1. Experimental arrangement for pheromone release and detection. Disk baffles of either 55 mm (green) or 22 mm (brown) diameter were placed 25 mm downwind of the release pipet to create turbulent plumes with broad dynamic range. An excised antennae was mounted on an electrode probe 25 cm downwind of the baffles, with a photoionization detector inlet (PID) located 1 mm farther downwind. The lower graph shows PID power spectra obtained during typical experiments with the baffles. Both spectra are shown on the same scale, which was normalized to the maximum value of the large baffle data. Arrow from baffle to antenna depicts wind direction.

FIG. 2. Frequency response and coherence functions for olfactory transduction in S. exigua (blue) and C. cautella (red) using pheromone blends. Frequency response data (points) are Bode plots of single experiments with solid lines showing fitted first-order low-pass filter functions (Equation 1) with parameters: $\alpha=1.40$, $\tau=13.2$ ms, $\Delta t=4.52$ ms ($S. exigua$) and $\alpha=0.21$, $\tau=62.2$ ms, $\Delta t=6.16$ ms ($C. cautella$). Coherence functions (lower) are for the same data. Information capacities calculated from coherence functions (Equation 2) were: 22.8 Bits/s ($S. exigua$) and 24.1 Bits/s ($C. cautella$).

FIG. 3. Mean and standard error values of fitted frequency response and information capacities from all experiments using pheromone blends. Numbers of experiments were: 12 for $S. exigua$ (blue) and 14 for $C. cautella$ (red). Amplitude and time constant were significantly different between the two moths ($P=0.0002$ and $P=0.0001$ respectively), whereas time delay and information capacity were not significantly different ($P=0.99$ and $P=0.42$ respectively).
**FIG. 4.** Mean and standard error values of fitted frequency response and information capacity parameters from experiments using different pheromone components. Ten experiments were used for each combination of species and component. No significant differences were seen between any of the fitted parameters for major and minor components stimulating *C. cautella* (red), whereas all of the fitted parameters were significantly different (P<0.05) between major and minor components for *S. exigua* (blue).

**FIG. 5.** LN cascade model of olfactory responses. Input signal, $x(t)$, consisting of pheromone concentration detected by the PID, was assumed to pass through a static nonlinearity approximated by a third-order polynomial function. Output of the polynomial, $w(t)$, then passed through a first-order low-pass filter with exponential impulse function, $g(u)$ to produce the EAG output, $y(t)$. Traces show typical PID and EAG signals from *S. exigua* (blue) and *C. cautella* (red) with model outputs obtained from fitting the LN cascade model (black) superimposed on the EAG signals.

**Fig. 6.** Fitted parameters from the LN cascade model. Upper graph shows all time constant values from fitting the model to pheromone blend responses, plotted versus time constants from frequency response functions of the same experiments. Data are shown separated by species and baffle size. The fitted line (black) has slope 1.13. Lower graph shows mean values of the fitted polynomial functions for *C. cautella* (red, 14 experiments) and *S. exigua* (blue, 12 experiments).
Figure 3

<table>
<thead>
<tr>
<th>Information capacity (Bits/s)</th>
<th>Time constant (ms)</th>
<th>Delay (ms)</th>
<th>Amplitude (x10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. exigua</td>
<td>50</td>
<td>20</td>
<td>1.0 (x10)</td>
</tr>
<tr>
<td>C. cautella</td>
<td>40</td>
<td>30</td>
<td>1.0 (x10)</td>
</tr>
</tbody>
</table>

* Indicates a significant difference.
Figure 4

Amplitude (x10)  Time constant (ms)  Delay (ms)  Information capacity (Bits/s)

S. exigua

C. cautella

Justus, Cardé and French
Figure 5

Justus, Cardé and French
Justus, Cardé and French

Figure 6