Controlled dynamic stimulation of *Drosophila* olfactory receptors

Julia Schuckel, Shannon Meisner, Päivi H. Torkkeli and Andrew S. French
Physiology and Biophysics, Dalhousie University, Halifax, NS, Canada

Introduction

Dynamic properties of sensory receptors limit the ranges of stimuli that can be detected, as well as the types and quantities of information transmitted to central nervous systems. Dynamic characterization can also place quantitative limits on the physiological components that could be responsible for sensory transduction and sensory information coding. Measuring the dynamic response of a sensory structure requires accurate control and measurement of its input stimulus, and a frequency range exceeding that of the system. This is difficult for chemoreceptors because of the nature of the stimulus. We have developed a new odorant stimulation system that overcomes these major limitations by providing controlled, wide bandwidth olfactory stimulation to insects or other animals. *Drosophila* were used for initial system development because they have become an important model of olfaction.

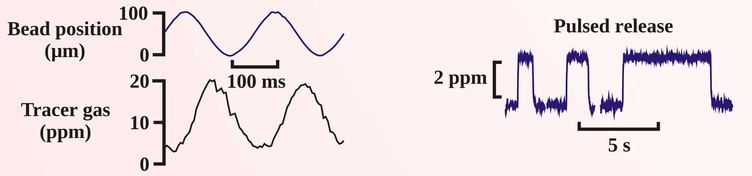
We used three major design criteria:

- Odorant concentration must be dynamically controlled to allow input-output systems analysis of olfactory receptor responses.
- Odorant concentration must be measured reliably at the sensillum.
- The frequency range of odorant concentration change must be wide enough to fully characterize dynamic sensory responses.

Methods

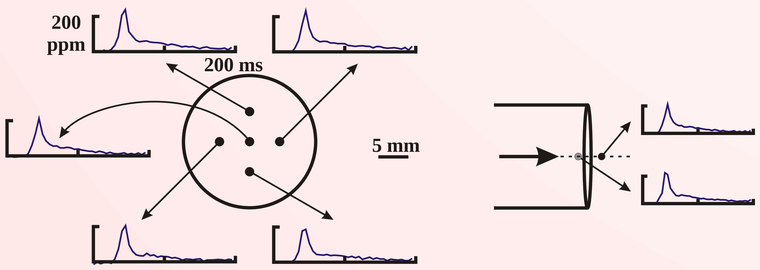
- Primary airflow from the fan passed through a laminar flow honeycomb into a circular plexiglas flow tube. The fly was at the far end of the tube, within 2-3 mm of the exit and 2-3 mm of the tube center line.
- Secondary air flow containing 1000 ppm propylene tracer gas flowed through an odorant cartridge into a Pasteur pipet with its tip located in the center of the circular tube.
- The pipet tip was variably occluded by a silicone elastomer bead driven by a mechanical stimulator and sensed by an infrared position detector.
- Flies (*Drosophila melanogaster*, Oregon R #2376) of either sex were used within two days of hatching.
- Odorants were natural fruit odors that excite *D*rosophila antennal olfactory receptors: butyl butyrate, isoamyl acetate, hexyl acetate and phenylethyl alcohol. *Drosophila* pheromone (Z)-11-octadecenyl acetate was also used.
- Tracer gas was measured by a photoionization detector with the tip of its probe located directly above and within 1 mm of the fly antenna.
- Reference glass microelectrode (~1 µm tip diameter) was inserted into one eye. A larger glass microelectrode (~20 µm tip diameter) was pushed against the distal tip of one antenna. Both electrodes were filled with *Drosophila* saline.
- Electroantennogram current was recorded by a List EPC-7 patch clamp amplifier.
- Frequency responses were calculated by direct spectral estimation during pseudorandom (white noise) stimulation.

Odorant concentration follows bead position

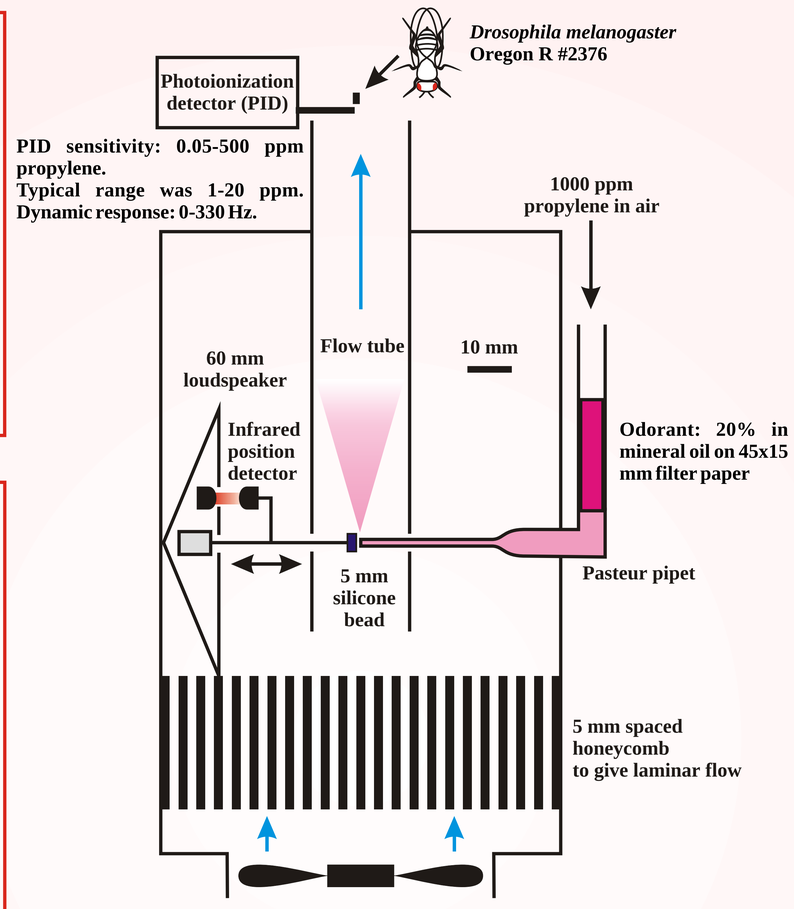


Tracer gas concentration with sinusoidal and pulsatile inputs. Note the delay between bead position and gas concentration caused by flow along the tube.

Odorant concentration has spatial homogeneity



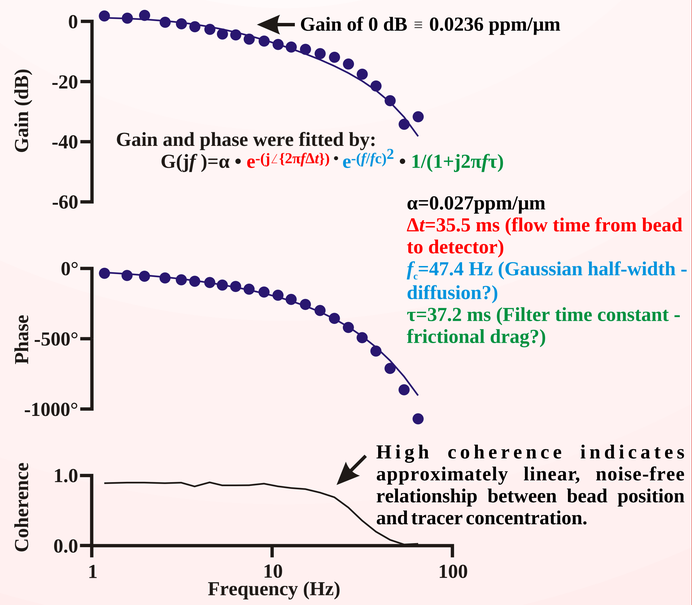
Odorant concentration near the animal following a brief pulse of gas from the Pasteur pipet at time zero. Tracer gas concentration was measured by the PID at different positions near the mouth of the flow tube. Note the agreement at different positions, except for delay due to flow along the tube. The Silicone bead position was actually moved randomly and the impulse responses estimated as Volterra kernels by the parallel cascade method.



60 mm fan - voltage controlled speed (4-12 V)

Stimulator frequency response

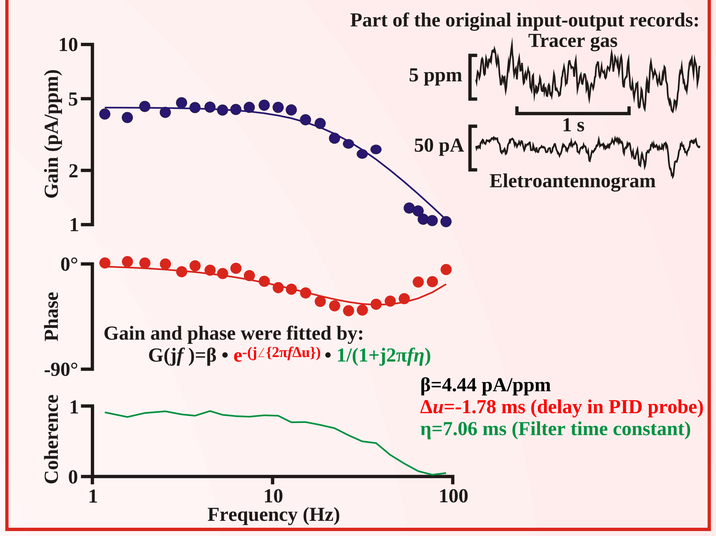
Frequency response was estimated by random (white noise) stimulation



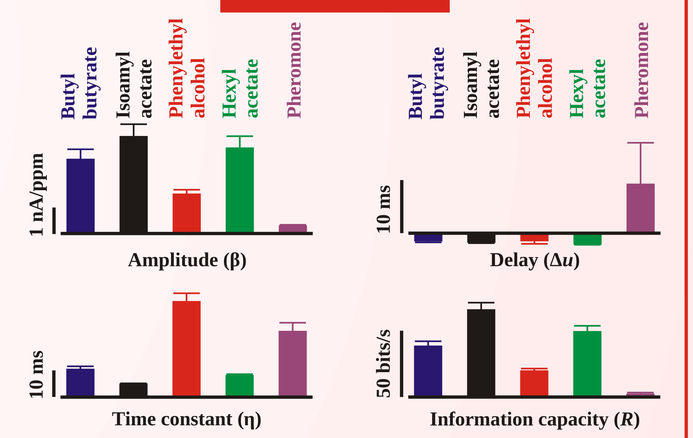
Frequency response between bead position and tracer gas concentration (measured by the PID at the mouth of the flow tube).

Frequency response of olfaction

Frequency response for Hexyl acetate estimated by random stimulation



Results



Mean ± SE values of fitted parameters for odorants (N=28) and pheromone (N=10). Information capacity was calculated from coherence ($\gamma^2(f)$) by:

$$R = \int \log_2(1/(1-\gamma^2(f))df$$

Conclusions and questions

- The new stimulation system allowed us to measure the frequency response of olfactory transduction over a range of at least 0 to 50 Hz
- Odorant concentration was reliable within at least 5 mm around the animal
- *Drosophila* antennogram responses were well-fitted by a first-order low-pass filter function, as was found previously for moth antennogram responses
- All odorant time constants and information capacities were significantly different from each other. Amplitude and delay values were not all different.
- Information capacity approximately followed response amplitude, indicating a constant background noise level in the antennogram.
- Fruit odors gave small negative delays, but pheromone gave a positive delay

Questions to be addressed:

- Which stage of olfactory transduction causes the low-pass filter response?
- Why does the time constant vary significantly for different odorants?
- Why does pheromone transduction involve a much longer delay?

Thanks to:
Dalhousie MEDICAL RESEARCH Foundation
Nova Scotia Health Research Foundation
CIHR IRSC

See this poster at: http://asf-pht.medicine.dal.ca/meetings