Dynamic characterization of *Drosophila* olfactory sensilla by random binary sequence stimulation
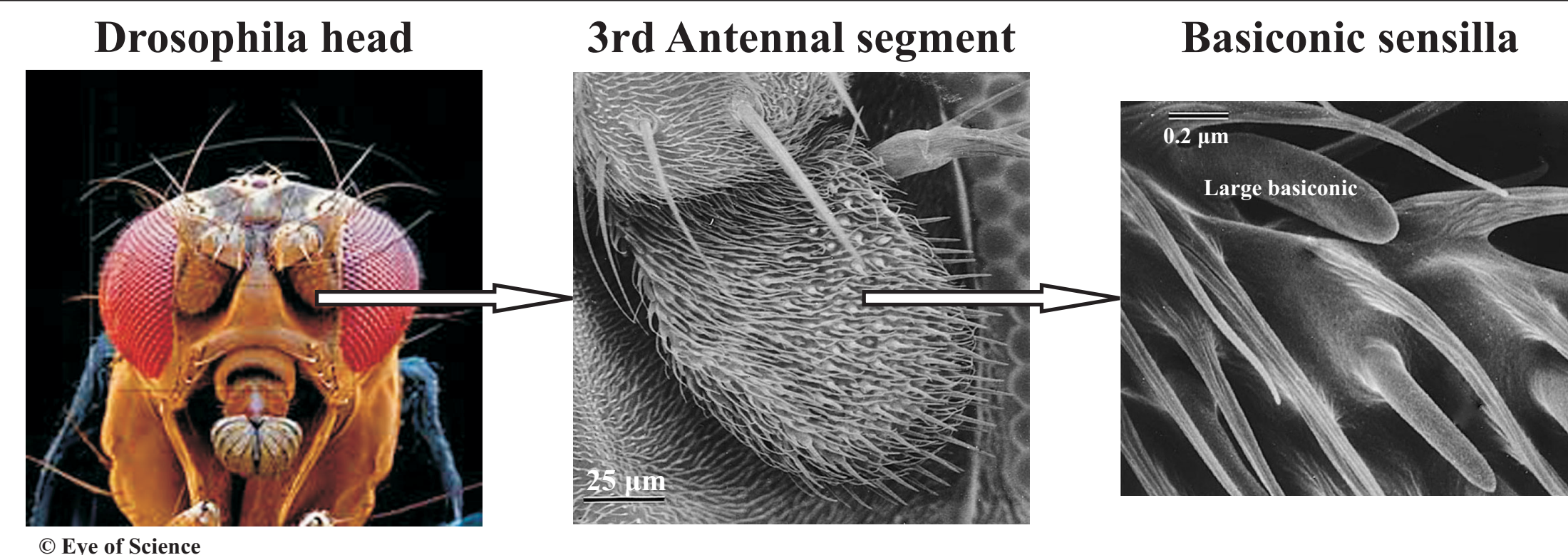
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1. Introduction

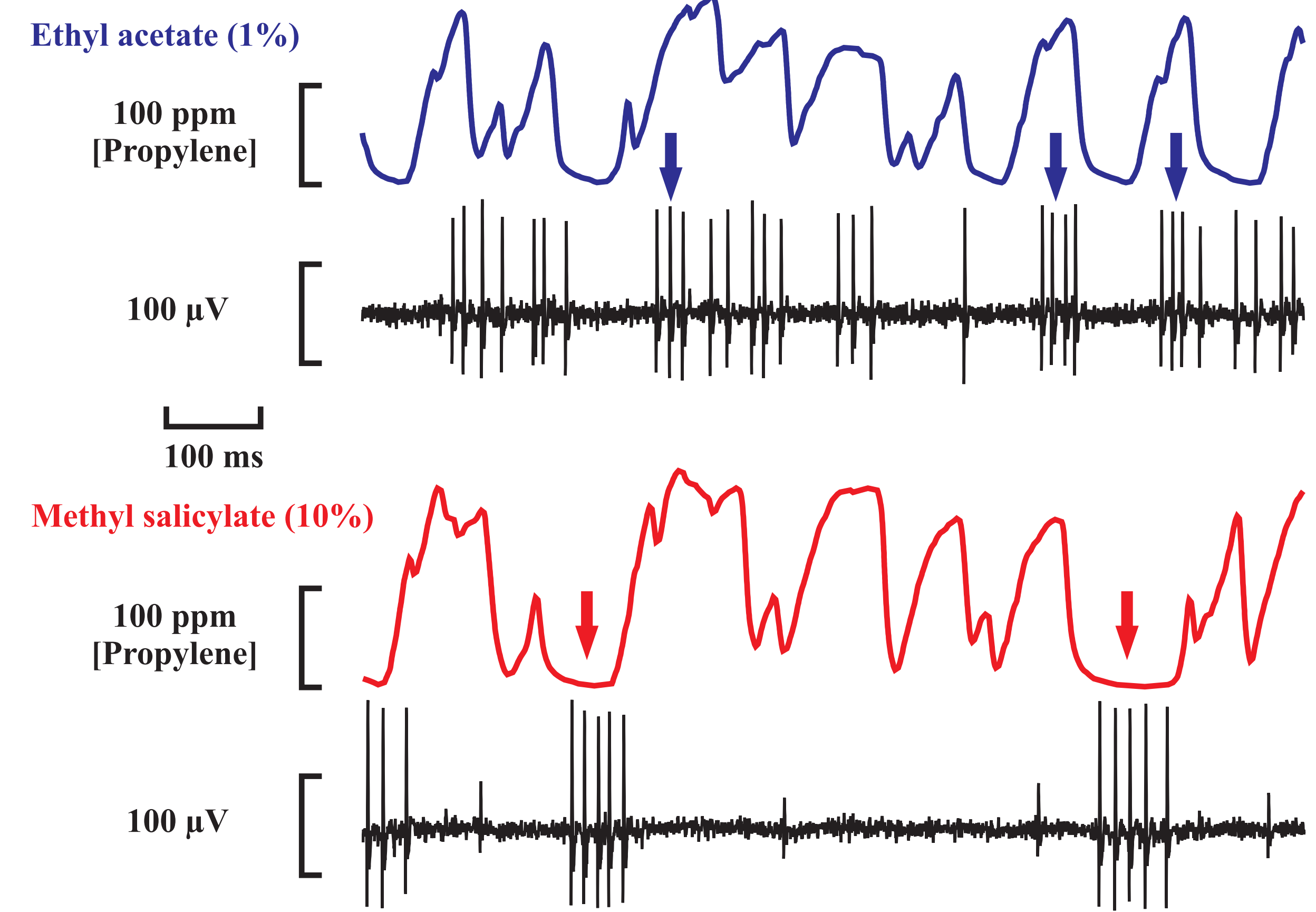**Drosophila head**   **3rd Antennal segment**   **Basiconic sensilla**

Three types of olfactory sensilla are located on the third antennal segment of *Drosophila*: basiconic, trichoid and coeloconic. Each sensillum contains 1-4 bipolar sensory neurons, with up to 3 different types of odorant receptor molecules (ORs) in the distal sensory dendrite, plus one type, OR83b, that is found in most neurons. Recent work indicates that OR83b combines with other OR molecules to produce odorant-activated cation channels, but the mechanisms and possible involvement of second messengers are unclear.

We are interested in the dynamic properties of chemotransduction in *Drosophila* basiconic antennal neurons. Detailed knowledge of receptor dynamics should provide important clues about the mechanisms of transduction, about the range of information that can be transmitted to the central nervous system, and about the contributions of olfaction to behavior.

Here, we report that different fruit odors produce two clearly separate response patterns in the same neurons: **positive** odors cause increased firing as their concentrations rise, while **negative** odors cause increased firing as their concentrations fall. The two odor groups also produce different, characteristic frequency response patterns. This clear separation of response patterns suggests the existence of two separate olfactory transduction mechanisms in olfactory sensilla.
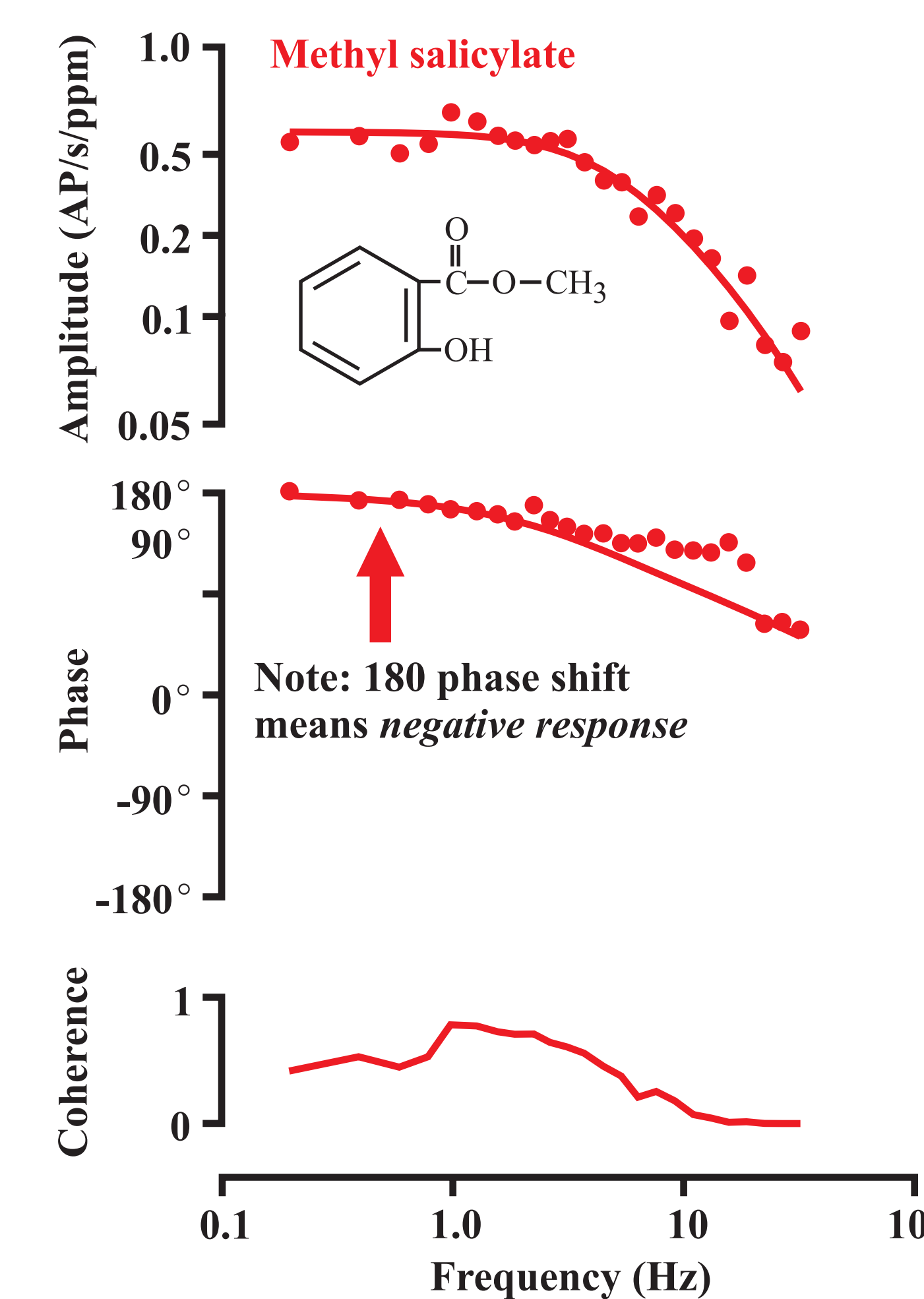
Scanning electron micrographs from: J.R. Riesgo-Escovar, W.B. Piekos and J.R. Carlson. (1997) J. Comp. Physiol. A 180: 151-160

2. Frequency response measurements

Primary air flowed through a 13 mm diameter, circular FEP tube. Secondary air containing 1,000 ppm propylene flowed over filter paper (45 mm x 15 mm) soaked in mineral oil plus dissolved odorants. Secondary air flow was switched on-off by a two-way solenoid valve, driven by maximum-length binary sequences, and entered the primary air flow via a 16 g hypodermic needle. *Drosophila* were in the center line of primary air flow. Tungsten electrodes recorded action potentials from single antennal basiconic sensilla. Propylene concentration was measured by a photoionization detector (sensitivity: 50-500,000 ppb propylene, frequency response: 0-330 Hz).

Drosophila melanogaster R#2376

Miniature photoionization detector (PID)

Propylene concentration

Tungsten electrode — Action potentials

Maximum-length binary sequence

Flow tube — Valve — Odorant on filter paper

Fan — 1,000 ppm Propylene in air

3. Two response types: positive and negative

Ethyl acetate (1%)

100 ppm [Propylene]

100 µV

100 ms

Methyl salicylate (10%)

100 ppm [Propylene]

100 µV

Single unit responses were of two types based on response polarity. This example shows a unit giving *positive* responses to Ethyl acetate (firing with *increasing* concentration, **arrows**), and *negative* responses to Methyl salicylate (firing with *decreasing* concentration, **arrows**).

4. Positive response - band-pass dynamics

Ethyl acetate

Amplitude (AP/s/ppm)

Phase

Coherence

Frequency (Hz)

$C_2H_5-\overset{O}{\overset{||}{C}}-O-CH_3$

Frequency response and coherence functions for randomly varying concentrations of Ethyl acetate (estimated from the propylene surrogate). Solid lines in amplitude and phase plots are fitted values of the complex band-pass function:

$$G(j\omega)=\alpha j\omega\tau_1/\{(1+j\omega\tau_1)(1+j\omega\tau_2)\}\quad (1)$$

where α is an amplitude parameter, τ_1 and τ_2 are time constants, ω is radial frequency and $j=(-1)^{1/2}$. A delay, Δt, was also included.

Similar responses were obtained for Ethyl butyrate and Hexyl acetate.

Analysis procedures

Frequency response and coherence functions were obtained by direct spectral estimation. Propylene concentration was the input signal and extracellular action potentials were the output signal. Action potentials were detected by a template matching algorithm and digitally filtered to 50 Hz maximum. Complex frequency response functions containing amplitude and phase information were fitted to filter functions by a minimum square error algorithm.

5. Negative response - low-pass dynamics

Methyl salicylate

Amplitude (AP/s/ppm)

Phase

Coherence

Frequency (Hz)

Note: 180 phase shift means *negative response*

Frequency response and coherence functions for randomly varying concentrations of Methyl salicylate (estimated from the propylene surrogate). Solid lines in amplitude and phase plots are fitted values of the complex, second-order low-pass function:

$$G(j\omega)=\alpha/\{(1+j\omega\tau_1)(1+j\omega\tau_2)\}\quad (2)$$

where α is an amplitude parameter, τ_1 and τ_2 are time constants, ω is radial frequency and $j=(-1)^{1/2}$. A delay, Δt, was also included.

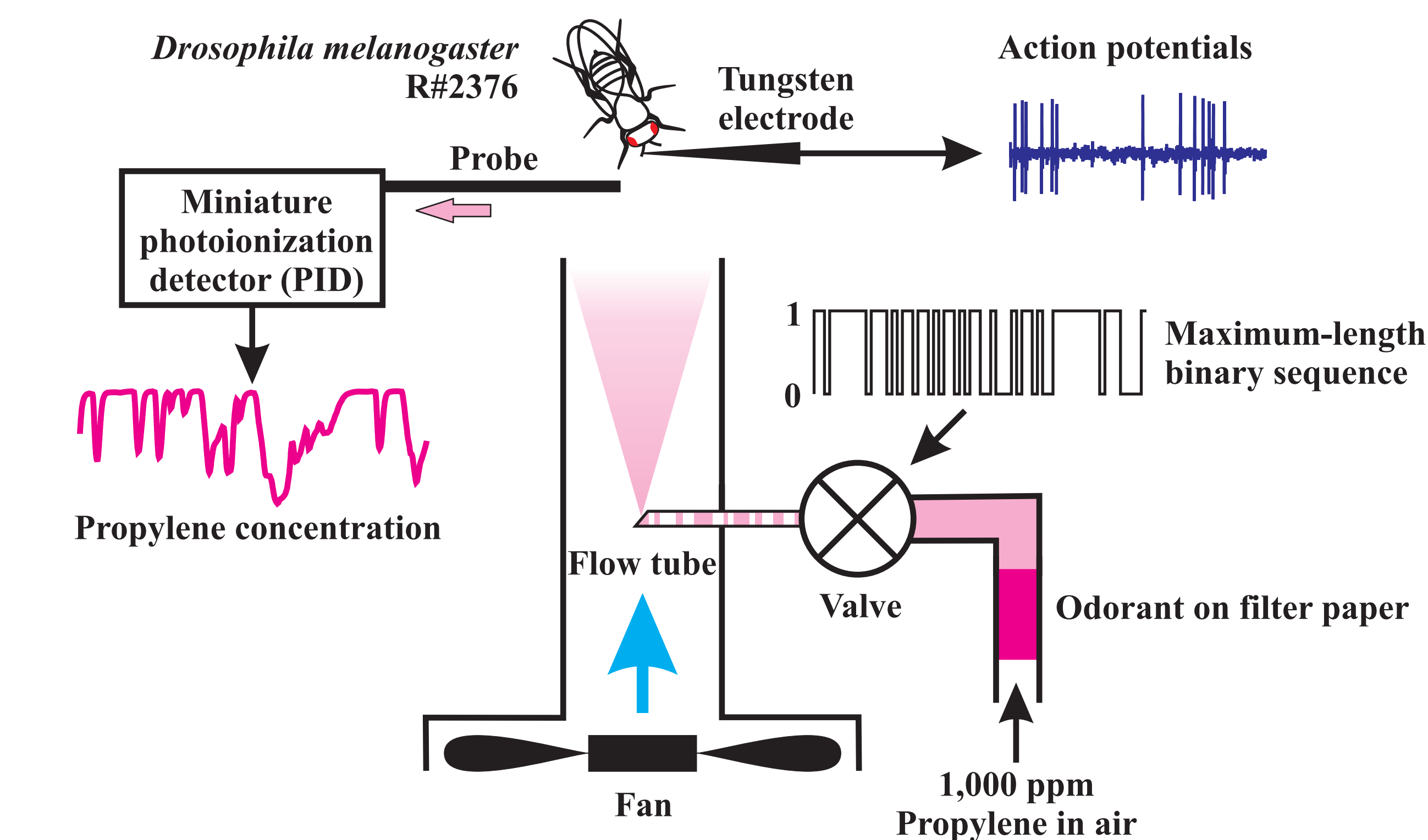Similar responses were obtained for Phenylethyl acetate.

Coherence and information capacity

The coherence function, $\gamma^2(\omega)$, measures the linear correlation between input and output signals. Values below unity occur if the system adds uncorrelated noise, or is nonlinear. Assuming that noise dominates, information capacity, R, can be estimated from:
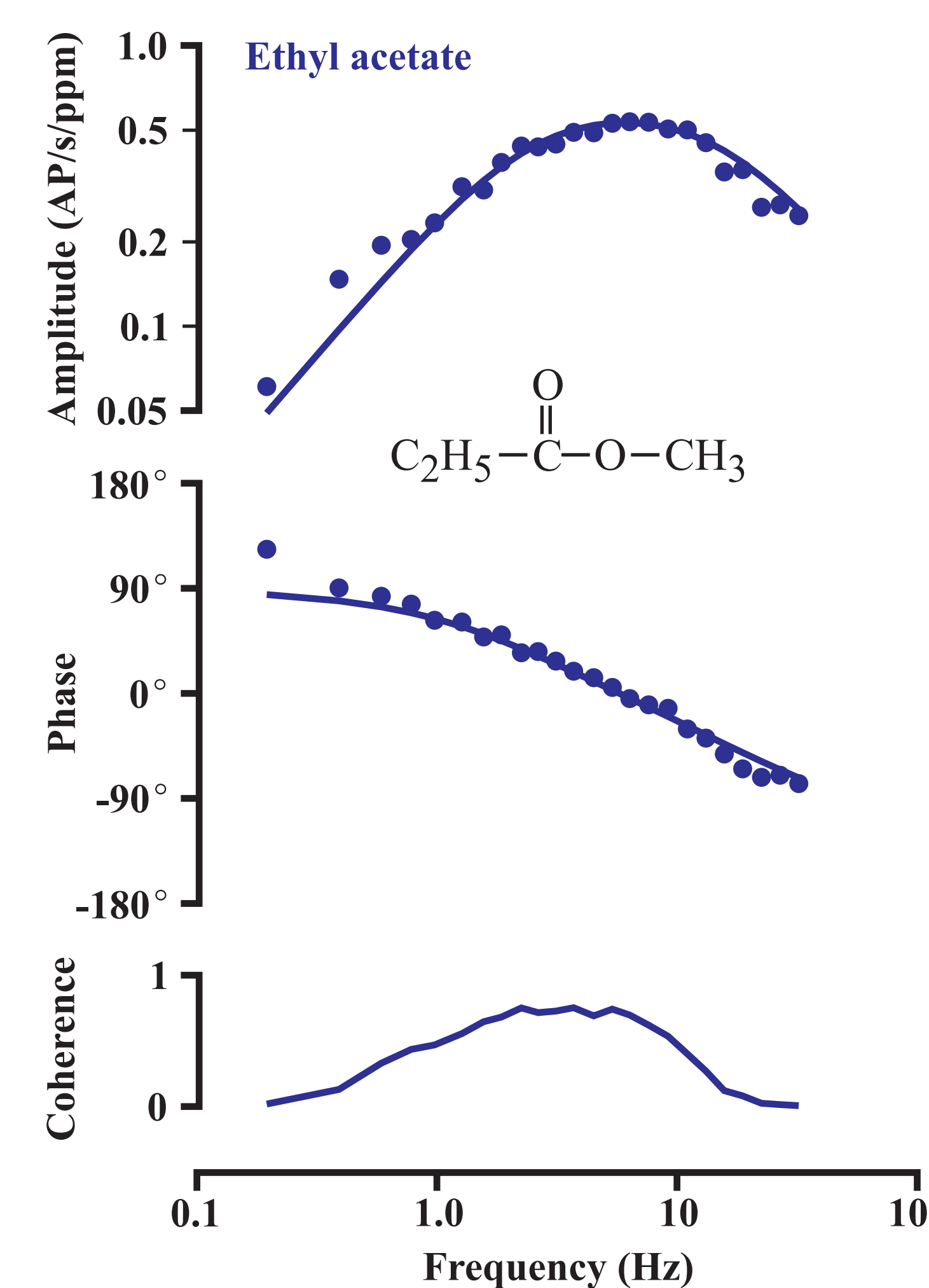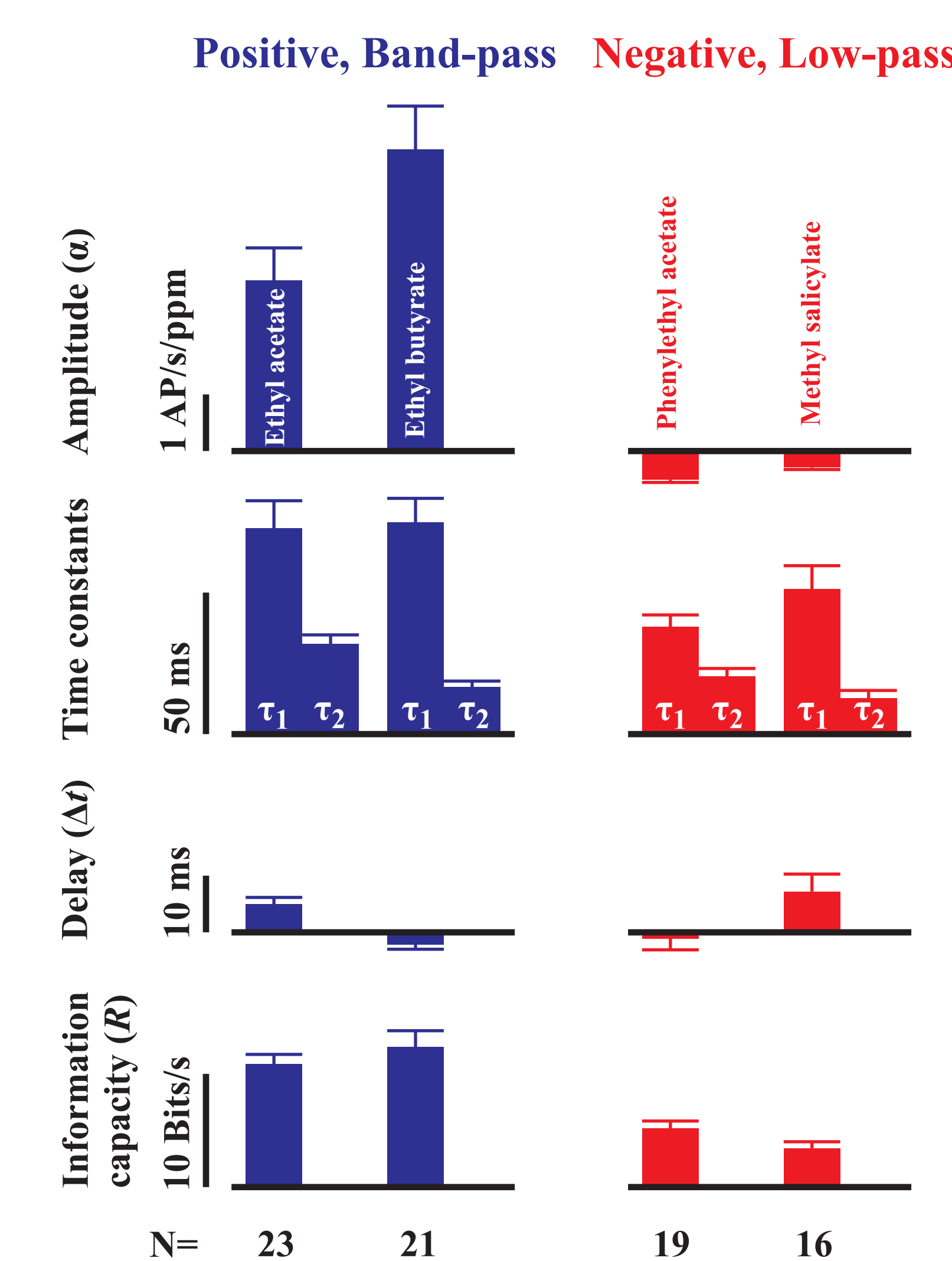
$$R=\int \log_2(1/(1-\gamma^2(\omega))\, d\omega \quad (3)$$

6. Fitted parameters

Positive, Band-pass   **Negative, Low-pass**

Amplitude (α) — 1 AP/s/ppm — Ethyl acetate, Ethyl butyrate / Phenylethyl acetate, Methyl salicylate

Time constants — 50 ms — τ_1 τ_2 τ_1 τ_2 / τ_1 τ_2 τ_1 τ_2

Delay (Δt) — 10 ms
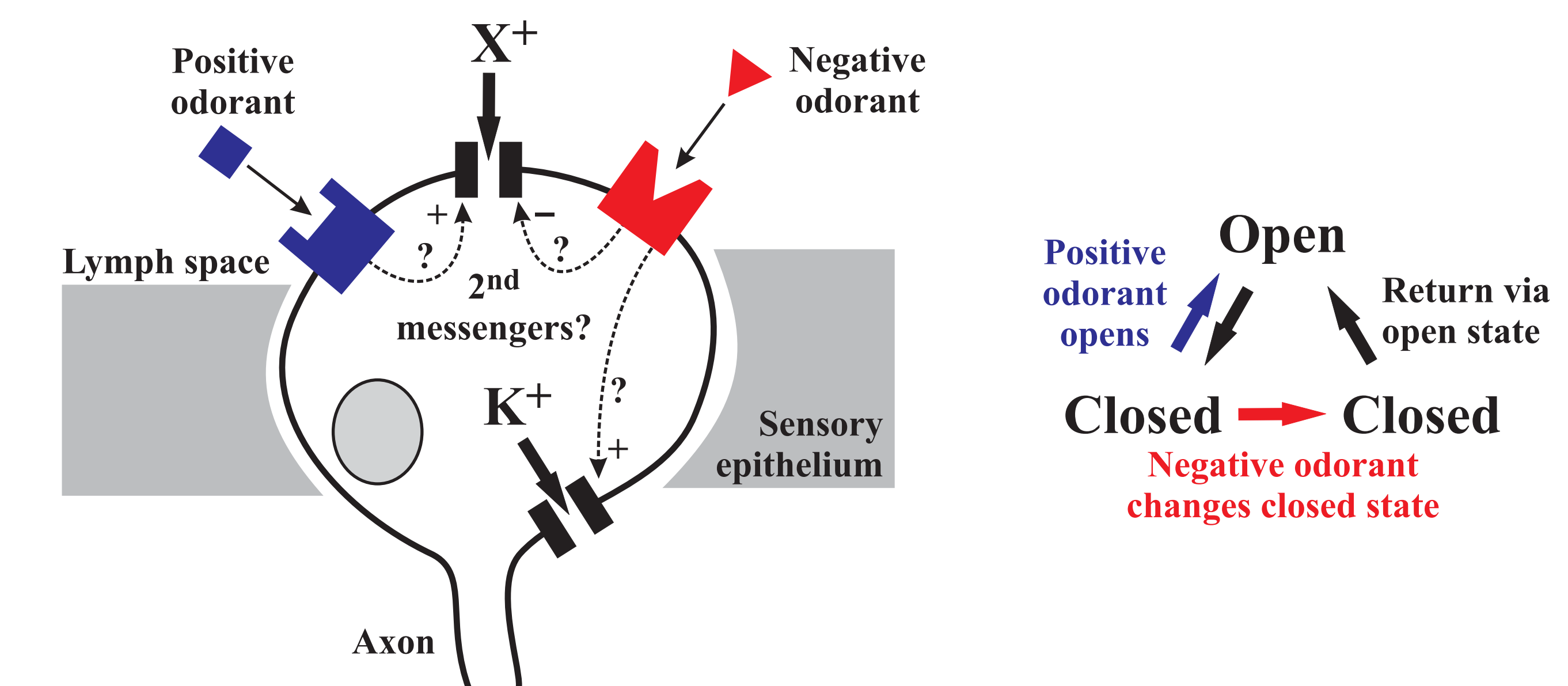
Information capacity (R) — 10 Bits/s

N=   23   21   19   16

Mean (±SE) values of parameters obtained by fitting Equations 1-3 to frequency response and coherence functions. Note that time constants τ_1, τ_2 have different meanings for band-pass and low-pass frequency responses.

Amplitude values are based on concentrations of propylene, not odorants, which are unknown. Ethyl acetate was loaded at 1%, all other odorants at 10%.

Delays include a contribution of about ~2 ms due to the location of the PID probe and flow time along the probe.

Information capacity values are about one tenth of similar measurements made in several mechanoreceptors, suggesting much lower signal-to-noise levels

7. Competition between two mechanisms?

Three aliphatic odorants gave positive, band-pass responses. Two aromatic odorants gave negative low-pass responses. Two different olfactory mechanisms?

Ethyl butyrate — Positive response

Phenylethyl acetate — Negative response

50% Ethyl butyrate + 50% Phenylethyl acetate — Negative response

Amplitude (AP/s/ppm)

Frequency (Hz)

Amplitude portions of frequency response functions from a single olfactory neuron stimulated with two odorants that produced positive and negative responses, respectively, and then with an equal mixture of the two compounds.

In three different experiments the response to the mixture was always similar to the response to the negative odorant alone. There was no consistent shift in any of the fitted parameters between the responses to the negative component alone and the mixture.

Responses to positive and negative odorants do not simply sum. Instead, there is competition between the two mechanisms and the negative response dominates.

8. Our suggestions for *Drosophila* olfaction

Positive odorant X^+ Negative odorant

Lymph space — 2nd messengers? — K^+ — Sensory epithelium

Axon

Positive odorant opens — Open — Return via open state

Closed → Closed

Negative odorant changes closed state

Three suggested hypotheses to explain our results:

1. (left diagram) Transduction channels are partially open in the absence of odorants. Positive odorants open them more (depolarizing), negative odorants close them (hyperpolarizing).

2. (left diagram) Positive odorants open transduction channels (depolarizing), negative odorants open K^+ channels outside the lymph space (hyperpolarizing).

3. (right diagram) Positive odorants open transduction channels (depolarizing), negative odorants put transduction channels into a different closed state that relaxes through an open state when odorant is removed (depolarizing).