POWER LAW ESTIMATES OF ODOUR THRESHOLDS

Matti Huotari, Vilho Lantto
Department of Electrical and Information Engineering,
Microelectronics and Materials Physics Laboratories,
University of Oulu,
FIN-90014 OULU, FINLAND
email: first name.surname

ABSTRACT

In this work a power-law dependence was obtained for the action potential rate recorded from olfactory receptor neurons (ORN) vs. odour concentration in measurements with metal microelectrodes. The exposure odour concentration (ppm), c_i, at each exposure was estimated according to the discrete multiple headspace extraction and dilution (DMHED) method. The action potential rate (pps) R_i in i^{th} odour exposure in a repeated exposure sequence was determined to follow a power law with an exponential decaying factor. The power-law exponent for ORNs of blowfly (*Calliphora vicina*) was determined to be 0.194 for 1-hexanol (HX), 0.323 for butyric acid (BA), and 0.064 for 1,4-diaminobutane (DAB). The odour threshold values were 0.01, 2.10 ppm, and $3.50*10^{-12}$ ppm, respectively.

INTRODUCTION

Clean air calls for accurate measurements. Many odorants are measured by analytical methods, e.g., chemical instruments in wet chemistry. Since biological odour relates to the psychophysical responses of humans to a mixture of odorants, individual odorants are not indicators of odour sensations. Organoleptic evaluations using the human ORN are considered to be the most valid procedure for odour evaluation and measurement. We are familiar with the odours of fresh and decaying fish, coffee, wine, meat, industrial odour emissions and so on, but it is impossible to define any exposure, e.g. odour exposure, without a measurement.

Insects have a good sense of olfaction for finding a mate and food. Detection is possible by the olfactory organs. The fast hearts of the olfactory organs are ORNs. Different insects have different odour chemicals for which they have specific ORNs which contain about fifty different receptor protein types on dendrites of each ORN. In insects, ORNs have been classified as generalist or specialist olfactory receptor neurons [1]. The specialist ORNs of blowfly (*Calliphora vicina*) is a case in point.

THEORY OF ODOUR EXPOSURES

In this study, we counted action potentials produced by an insect ORN to evaluate the olfactory exposure intensity, namely odour concentration.

The stimulus amount of odour exposure was determined as follows. An initial amount of stimulus compound in the filter paper m_0 causes a concentration c_1 in the pipette volume V during evaporation of a fixed time, 1 s. In a repeated exposure sequence the concentration $c_i=m_i/V$ in the i^{th} exposure follows according to the DMHED method an exponential expression because of diffusion

$$c_i = c_1 e^{-\beta\cdot(i-1)}, \quad i=1,2,3, \ldots, n \tag{1}$$

where $c_1=m_1/V$ is the concentration in ppm (part per million) of a stimulus compound in the first (i=1) exposure, m_i the amount of stimulus matter extracted in the i^{th} exposure and β

the exponential decaying coefficient. It is possible to obtain an expression for the initial amount of m_0 by applying the sum rule for the geometric series of the equation (1)

$$m_0 = \sum_{i=1}^{\infty} m_i = V \sum_{i=1}^{\infty} c_i = V \sum_{i=1}^{\infty} c_1 e^{-\beta\cdot(i-1)} = V \frac{c_1}{1-e^{-\beta}} \tag{2}$$

This gives an equation for c_1

$$c_1 = \frac{m_1}{V} = \frac{m_0}{V}(1-e^{-\beta}) \tag{3}$$

The factor $e^{-\beta}$ means now the partition fraction of the stimulus compound between a support material and air in the exposure. A fitting procedure for the exposure concentrations based on a power-law dependence of the action potential rates on the odorant concentration was used to calculate values for the decaying coefficient β.

In the experiments, the action potential rate R_i in pps (pulses per second) of blowfly ORNs follows an exponential expression with an exponential decaying factor α^* during the i^{th} exposure in a repeated exposure sequence to an odorant compound, see also Figure 2.

$$R_i = R_1 e^{-\alpha^* \cdot (i-1)}, \ i=1,2,3, \ldots \tag{4}$$

By taking logarithm on the equation (4), we receive

$$\ln\left(\frac{R_i}{R_1}\right) = -\alpha^* \cdot (i-1) \tag{5}$$

From equation (1) it is also possible to solve by the similar way

$$-(i-1) = \frac{1}{\beta}\ln\left(\frac{c_i}{c_1}\right) \tag{6}$$

The insertion of the term -(i-1) from equation (6) to the equation (5) gives a new equation

$$\ln\left(\frac{R_i}{R_1}\right) = \frac{\alpha^*}{\beta}\ln\left(\frac{c_i}{c_1}\right) \tag{7}$$

Equation (7) is a power-law dependence (according to Stevens' law) for the action potential rate on the stimulus concentration provided that a flight time (about 80 ms) of the odour exposure is eliminated in counting an action potential rate response. By taking antilogarithm on the both sides of the equation (7) gives

$$\frac{R_i}{R_1} = \left(\frac{c_i}{c_1}\right)^{\frac{\alpha^*}{\beta}} \tag{8}$$

The corresponding continuous equation is

$$\mathrm{R}(\mathrm{c}, a, n) = a \cdot \mathrm{c}^{n} \tag{9}$$

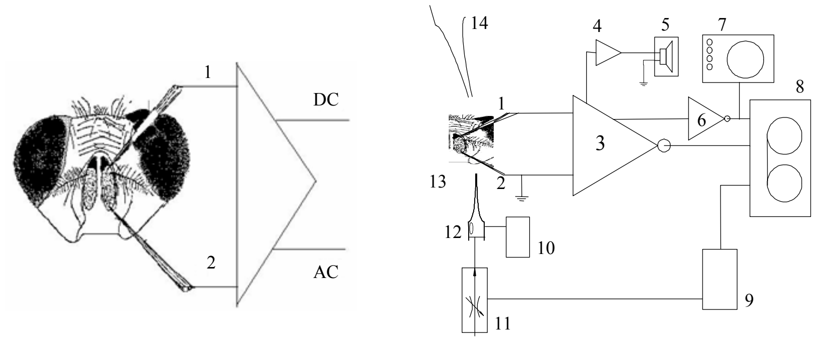
where a term *n* is an olfactory exponent and a term *a* is a proportional coefficient.

Equation (9) is similar to the Stevens' power law for the sensory function in the psychophysics [2]. An exponent symbol *n* depends on odour compound, gender, age and time of day and the term *a* is a proportionality coefficient. This equation gives a straight line on $\log_{10} - \log_2$ co-ordinate axes with the slope *n* and the intercept *a*.

For unifying the information of human experience with electrophysiological measurements from insect ORNs, we need to be able, at least, to connect some basic odours with ORN action potential rates to odours, like diamine (1,4-diaminobutane), alcohol (1-hexanol) or an organic acid odour (butyric acid). This kind of research could support the Stevens' power law which fits very well to the results of insect ORNs as odour sensors for finding the odour absolute threshold, so called olfacty. Olfacty is then lowest concentration which is just detectable.

MATERIALS AND METHODS

The measurement system is shown for single sensillum recordings (SSR) in a blowfly antenna in Figure 1. The blowfly (*Calliphora vicina*) ORNs were exposed to three compounds at a time sequence.



(a) (b)

Figure 1. (a) The measurement setup for single sensillum recordings (SSR) in a blowfly antenna. The electrode 1 is the measuring electrode and electrode 2 for the reference electrode, AC is for alternating signal (action potentials) and DC for direct signal (EAG). (b) Schematic drawing of the odour exposure measurement system for insect ORNs. Measuring (1) and reference (2) electrodes, microelectrode amplifier (3), audio amplifier (4), loudspeaker (5), instrument amplifier (6), oscilloscope (7), DAT recorder (8), electronic exposure control unit (9), rotameter (10), magnetic valve (11), filter paper in the Pasteur pipette (12), insect antenna (13) and clean air flush flow (14).

In a single sensillum recordings (SSR) the same ORN could respond to some odours by excitation and to others by inhibition. Single cell ORN activity is recorded with the measurement setup shown in Figure 1 (a) and (b). The excitatory response of the blowfly ORNs are predominated in odour responses, each ORN being strongly excited by certain odours, e.g. HX in this case. The sharp microelectrode 1 is inserted into the sensilla “forest” randomly (Figure 1 (a)). Based on the measured signal to the response of odour exposures, it is possible to decide the actual location of the active ORN (Figure 1 (b)) [3].

RESULTS AND DISCUSSION

Figure 2 shows the action potential rate of a blowfly ORN as a function of the exposure number to HX (initially 50000 ng in the filter paper) of repeated exposures 2-10 in the sequence. The first exposure is left off from the data fitting, since the evaporation time in the pipette was different from the constant evaporation time period (1 s) in the exposure sequence. The reason for this difference was the installation of the filter paper inside the pipette.

The exponential decrease of the action potential rate as a function of the exposure number in the exposure sequence is in agreement with equation (4) but also with equation (1). This kind of odour delivery system, an olfactory stimulator, interferes the minimum an ORN under study, however, changing of a new pipette in to the olfactory stimulator has to be carefull. By this way it is possible to complete the exposure sequences in Figure 2 and 3 where there are over 40 odour exposures in five sequences.

After the odour exposure measurements, the fitting procedures produce the olfactory exponent and the intercept values with their mean errors which are also shown in Figure 3. Based on these values it is possible to calculate futher the thresholds and their relative errors.

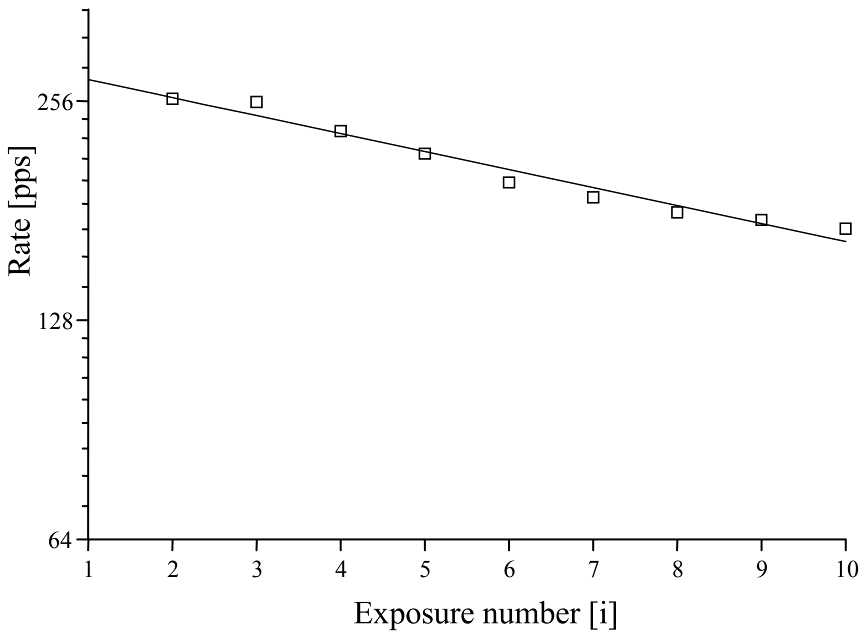


Figure 2. Action potential rate of a blowfly ORN as a function of the exposure number to HX in a repeated exposure sequence (i=2, …, 10). Initial amount of HX in the filter paper was 50000 ng.

Figure 3 shows the measurement results with one ORN of an individual blowfly. The results from the three exposure sequences with the same initial amount of 50000 ng HX in the filter paper are in a close agreement [4]. The decaying action potential rates at repeated exposures in the sequences relate to decreasing HX through the exponential constant of decaying odour concentration. In Figure 3 the initial amount of 100000 ng HX did not caused an exponential decreasing because of a too high amount of matter.

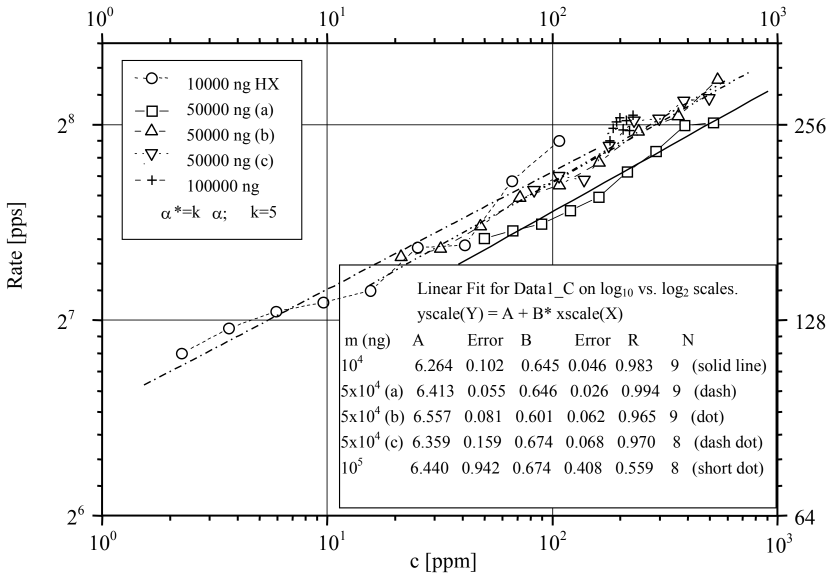


Figure 3. Action potential rates of a blowfly ORN vs. concentration of HX estimated by the DMHED method (α*=kα with k=5) for the initial amounts of HX in the filter paper of 10000, 50000 (three sequences (a), (b) and (c)) and 100000 ng together with linear fits to the experimental values in the lower inset [4].

Also the decreasing was not caused by a simultaneous decaying responsiveness of the preparation, insect ORNs neural activity. This blowfly ORN did not respond to the DAB odour. Many different ORNs were active to HX, and had action potential rates at about the same level at exposures to HX. The standardized exponent is 0.194 for HX. Psychophysical studies on olfaction have dealt with different aspects of human odour perception: odour quality, odour threshold and the supra-threshold intensity function. Based on the theory of the Stevens' power law it is possible to relate the intensity of the perception to the stimulus concentration and to derive the psychophysical exponent and the threshold concentration c_o

$$c(R_o, a, n) = c_o = \left(\frac{R_o}{a}\right)^{\frac{1}{n}} \tag{10}$$

where R_o is the spontaneous activity (which is 35 pps typically).

The exponents for ORNs of blowfly (*Calliphora vicina*) are 0.194 for HX, 0.323 for BA, and 0.064 for DAB. The thresholds, c_o, are 0.01, 2.10 ppm, and $3.5*10^{-12}$ ppm, respectively, calculated by the equation (10) based on the SSR method. The values of 0.39 and 0.33 have been reported for 1-hexanol and butyric acid, respectively, in psychophysics based on electroantennograms (EAG) [5].

This is the Stevens' power law with standardized olfactory HX, BA, and DAB exponent for a blowfly ORN. We can also determine the total errors for the threshold values received by a total error estimate according to the derivative

$$dc_o = \left(\frac{\partial c_o}{\partial R_o}\right) dR_o + \left(\frac{\partial c_o}{\partial a}\right) da + \left(\frac{\partial c_o}{\partial n}\right) dn \tag{11}$$

The equation (11) results the relative total error for the threshold concentration

$$\left|\frac{\Delta c_o}{c_o}\right| = \frac{1}{n}\left(\left|\frac{\Delta R_o}{R_o}\right| + \left|-\frac{\Delta a}{a}\right| + \left|-\left(\ln\left(\frac{R_o}{a}\right)\right)\frac{\Delta n}{n}\right|\right) \tag{12}$$

In the equation (12) we get Δa and Δn from the fitting of the data (Figure 3) and ΔR_o from the instrument accuracy value in action potential rate measurement and R_o is the spontaneous action potential rate. The relative total errors for the odour threshold concentrations, $\Delta c_o/c_o$, are 1.06 %, for HX, 1.76 % for BA, and 1.40 % for DAB, respectively, calculated by the equation (12). The error originates both from the power model, the accuracy of fitting, the measurement technique and the spontaneous conditions of the insect olfactory receptors (R_o=35 pps).

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