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# Characteristics of Electroantennogram Parameters for the Detection of Odorants

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**Abstract** Electroantennogram (EAG) can be applied to detect odorants since insects have highly specialized olfactory receptors inside their antennae. The characteristics of EAG parameters were investigated for the quantitative measurement of a general odorant using ammonia as a model odorant. The antennae of male silkworm moth, *Bombyx mori*, were used for the EAG. The electrical signal curves generated from a pair of antennae originating from the same silkworm moth were never identical; however, they exhibited a typical type I or type II characteristic curve pattern for every pair of antennae. The correlation between the EAG parameters and the ammonia concentration was analyzed for the type I and type II antennae. The stability of each parameter was also investigated for each type of antenna. The results show the possibility of the quantitative measurement of general odorants using the EAG technique.

**Key words:** Electroantennogram, *Bombyx mori*, EAG parameter, stability, olfactory receptor

Sense of smell is the most efficient means to communicate with others and interpret their surroundings [4, 11, 15, 17]. In fact, most animals rely on the olfactory sense to identify food, predators, and mating partners. The olfactory system has the remarkable capacity to discriminate among a wide range of odor molecules. The mammalian olfactory system has an ability to detect odorants at a low concentration and discriminate among thousands of distinct compounds. Recently, interest in olfaction research has increased due to its potential industrial applications. Many researchers have attempted to understand the molecular mechanisms that account for the sensitivity and specificity of smell perception [14]. Olfactory perception consists of many complex biochemical and electrophysiological processes.

The olfactory system consists of four main structural regions [12], each specialized for one or more distinct functions and related to one or more of the four main extracellular environments. The first structure consists of cilia, that is contained in an extracellular mucus, primarily where signal transduction takes place. This ciliary structure provides an increased surface area for effectively capturing the odorant molecule [3]. The second structural region consists of the neuronal cell body (soma) and dendrite, which provides for two main functions: electrical impulse generation and the regulation of gene expression to control cell differentiation and maturation. The third structure consists of the axon that mediates the electrical impulse transmission from the olfactory epithelium to the olfactory bulb. The fourth region is the olfactory bulb consisting of glomeruli and neurons. The neurons transmit signals from the glomeruli to the olfactory cortex, where the signals are processed for odor discrimination.

The olfactory structure and odorant detection process are similar in vertebrates and insects. However, compared with a mammalian olfactory system, an insect system has some unique features. The anatomy is convenient for isolating the antenna which is the olfactory organ. Many researches have been carried out using insect cells [5-10, 13]; however, the whole insects or their parts have not been extensively investigated for biotechnological applications. The insect antenna is one of the useful materials for this purpose. Electrical signal recording techniques are well established, and electroantennogram (EAG) signals can be recorded from the whole antenna as small voltage shifts between two electrodes which are inserted at the base and tip of the antenna [1, 16]. The investigation of EAG using insect antennae has been focused primarily on the measurement of insect pheromones and plant volatiles. However, it was shown that EAG can also be applied to detect general odorants [18]. In this study, the characteristics and stability of EAG parameters were investigated for the quantitative measurement of a general odorant.

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### Analysis of EAG Response Shape

Adult male silkworm moths, *Bombyx mori*, were kindly provided by Dr. Sam-Eun Kim, Department of Sericulture and Entomology, National Institute of Agricultural Science and Technology, Suwon, Korea. Antennae were cut off from the three- to four-day-old moth using microscissors. It was then placed on an antenna holder which consisted of two 1.7 mm diameter wells filled with 0.1 M KCl solution. The recording electrode was placed in the well with the base of the antenna submerged, while the reference electrode was placed in the other well with the tip of the antenna submerged. The antenna holder containing the two wells was then placed in a cylindrical plastic casing with a diameter and length of 60 mm and 250 mm, respectively. The air flow rate was constantly maintained at 30 l/min using a suction pump and various concentrations of the $NH_3$ gas as the model odorant were injected into the air stream using a syringe. The electrical signal was preamplified (IX1, Dagan Co., Minneapolis, U.S.A.), displayed on an oscilloscope (TDS 220, Tektronix Inc., Wilsonville, U.S.A.), and stored on a computer hard disk.

The intensity of the amplifier was measured using a step input of 0.067 V. The amplified output (7.39 V) was 110 fold higher than the input voltage (data not shown). This information was used for the calculation of original electrical signal generated from the insect antenna. Figure 1 shows the typical EAG responses stimulated by the $NH_3$. An insect has two antennae and the electrical signals generated from the two antennae resulted in different shapes. One signal curve showed a faster behavior than the other. The fast-behaving curve showed a fast rise and decay, and the decayed signal usually passed the original base line in this type of curve. In some cases, the right-side antenna generated a faster curve pattern than the left-side one, and vice versa in other cases. The curve pattern showing the slower behavior was denoted as type I, and the other pattern was denoted as type II. The signal curves generated from a pair of antennae originating from the same silkworm moth were never identical; however, every curve for each silkworm moth exhibited a typical type I or type II characteristic curve pattern.

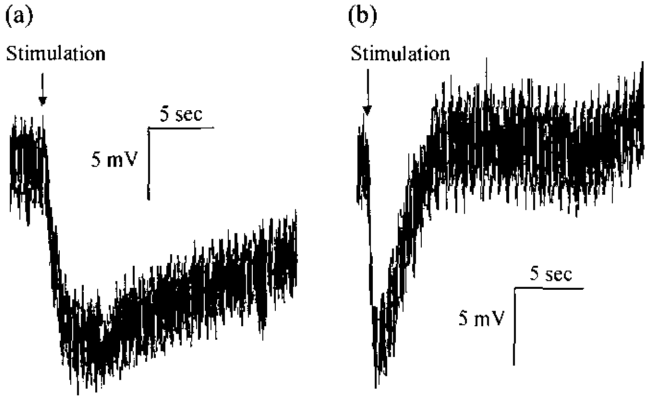

**Fig. 1.** Typical EAG generated by $NH_3$ stimulation.
(a) type I, (b) type II. The signal curves generated from a pair of antennae originating from the same silkworm moth were never identical; however, they exhibited a typical type I or type II characteristic curve pattern for every pair of antennae.

The response curves were divided into two parts, first the rising part and second the decaying part, that is, the regions before and after reaching the peak apex, respectively. The slow-behaving curve (type I) was more suitable for analyzing the rising part since the rise in the fast-behaving curve was too fast for an analysis. Whereas the fast-behaving curve (type II) was more suitable for analyzing the decaying part, the decay was rather sharp and clear in this curve.

### Analysis of EAG Parameters and their Stability

The original responses shown in Fig. 1 have much noise, and the noise makes the parameter analysis difficult. Thus,

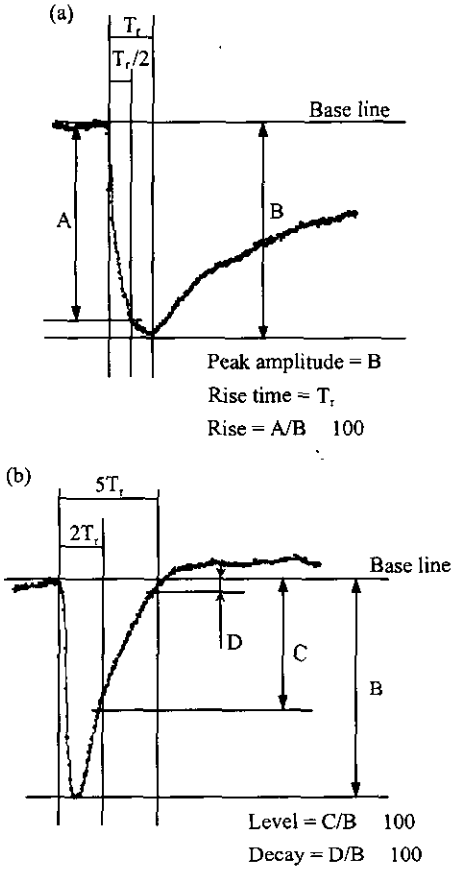

**Fig. 2.** EAG parameter analysis.
(a) type I, (b) type II. The peak amplitude (B) is the peak height from the base line. The rise time ($T_r$) is the time at which the signal reached the lowest point. The rise (A/B × 100) is the ratio between the value after the first half of the rise time (A) and the peak amplitude (B). The level (C/B × 100) is the ratio between the value at 2 $T_r$ (C) and the peak amplitude (B). The decay (D/B × 100) is the ratio between the value at 5 $T_r$ (D) and the peak amplitude (B).

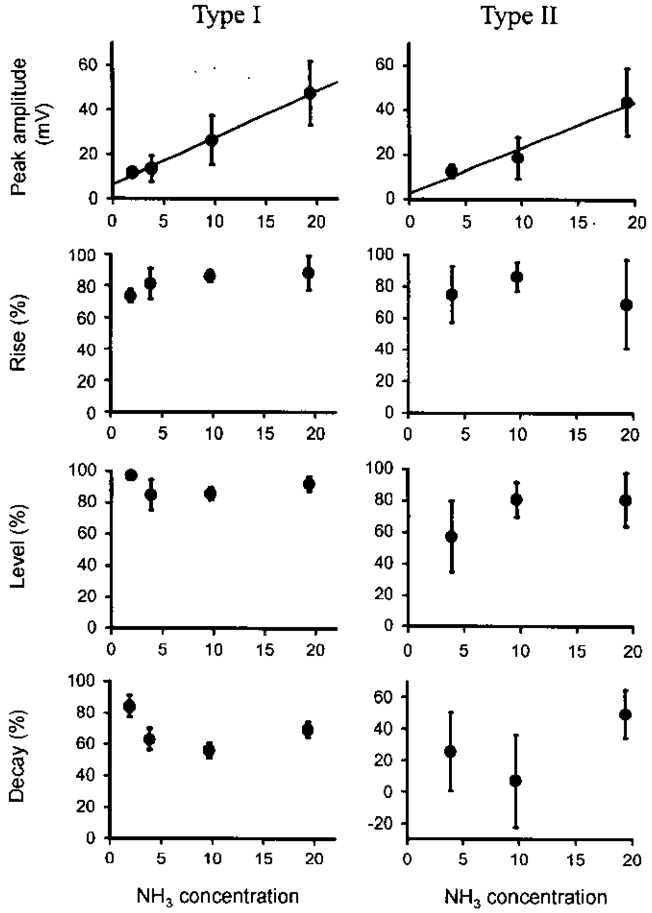

**Fig. 3.** Correlation between EAG parameters and $NH_3$ concentration for type I and II responses.
The peak amplitude increased linearly with the $NH_3$ concentration for both type I and II antennae.

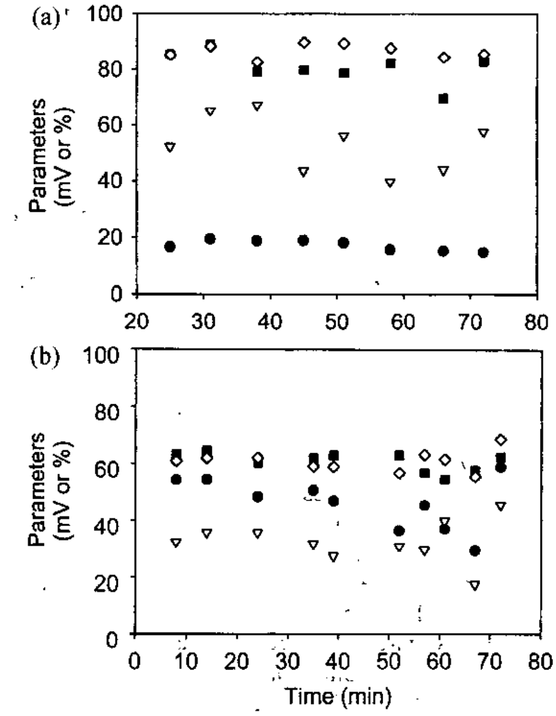

**Fig. 4.** Stability of various parameters.
(a) type I ($NH_3$: $9.69\times10^3$ ppm), (b) type II ($NH_3$: $1.94\times10^4$ ppm). ●: Peak amplitude (mV), ■: Rise (%), ◇: Level (%), ▽: Decay (%).

the noise was removed by taking an average of seven data sets, and the results are shown in Fig. 2, where the EAG parameters such as peak amplitude, rise time, rise, level, and decay are defined. The peak amplitude is the peak height from the base line. The parameters related to the rising parts are rise time and rise, while those related to the decaying part are level and decay. The correlation between the parameters and the $NH_3$ concentration for type I and type II antennae is shown in Fig. 3. The peak amplitude increased linearly with the $NH_3$ concentration and similar results were obtained from both type I and II antennae. The peak amplitude is the most widely used parameter in EAG research for the detection of insect pheromones. However, no correlation could be found between other parameters and the $NH_3$ concentration. Although only the peak amplitude is suitable for measuring odorant concentration, other parameters could be used for discriminating between different odorants.

After separating from the body, an antenna is only active for a limited time period. Figure 4 shows the stability of the parameter values for type I and type II antennae. It has been reported that the lifetime of an antenna after separation is usually between 3- 30 min. However, this figure shows that the peak amplitude and other parameters are stably maintained longer than the reported ones.

Various kinds of electric noses have been developed by mimicking the biological olfactory system. The principle of the electric nose is a statistical mapping technique based on the fundamental olfactory mechanism. It consists of various gas sensor arrays. The gas sensors used in the electric nose include a metal oxide, piezoelectric device, conducting polymer, and optical fiber [2]. The electric nose then depends on the electrical signals generated from various odorant-binding materials. Accordingly, many different sensing materials are required to discriminate among the wide range of odor molecules. In contrast, a biological olfactory system has thousand different receptor cells in it. Therefore, if a biological system such as an insect antenna is developed instead of using chemical materials, this would overcome the limitations presented by the electric nose.

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