PROPERTIES OF ELECTROANTENNOGRAMS IN FLIES

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Summary
By varying the positions of electrodes on the antenna, it was revealed that EAGs from globular antennae measure the summated receptor potentials over the cuticula of olfactory receptor neurons near the electrode.
Two effects of acids were found: 1. Using low doses of acids, EAGs reflect the physiological responses of olfactory receptor cells. 2. High doses of strong acids induce electrochemical potentials of the electrodes that vary in strength and polarity, depending on unknown factors. Therefore care should be taken in interpreting responses to these stimuli.

INTRODUCTION

The electroantennogram (EAG) has been used in a variety of insects as a convenient screening procedure for pheromones and odours that could be of importance for the animal. Most work has been done on the filamentous antennae of moths and it has been shown that the size of the EAG is proportional to the number of olfactory sensilla between the electrodes (Nagai, 1981; Mayer et al., 1984; White, 1990). Each olfactory cell can be considered to become a small dipole during stimulation, because the receptor potential depolarises only the dendrite of the cell. The serial arrangement of the sensory cells causes a summation of the receptor potentials of many cells between tip and base of the antenna (Figure 1a, after Kaissling, 1971). The shape of the antennae of house flies and several other Dipterans, however, is not filamentous but merely globular and EAGs seem to be formed in a different way. In this study we investigated technical properties of EAGs.
Many insects are attracted by acids. When measuring EAG-responses to acids, sometimes extreme values were found. Meijerink (1999) showed that using tungsten electrodes, electrochemical potentials were formed upon stimulation with acids, that interfere with physiological responses. We investigated this effect using glass electrodes.

MATERIALS AND METHODS

Mature, female house flies (*Musca domestica* L.) were used. An intact fly was immobilized in a Finn-pipette with its head protruding out of the tip. EAGs and activities of individual odour receptor cells were recorded with glass micro pipette/Ag-AgCl electrodes filled with Ringer solution for both the recording and the indifferent electrode. Stimuli were prepared by dissolving 1 mg 1-octen-3-ol, or 10 mg and decadic dilutions of the acids in 25 ml silicon oil. This was pipetted onto a filter paper inside a Pasteur pipette, that served as an odour cartridge. Air was blown for 0.2s through an odour-loaded Pasteur pipette into an airflow flowing over the antennae.

RESULTS AND DISCUSSION

When a recording electrode is inserted into the tip of an antenna, and the indifferent electrode near the base of the antenna, it is difficult to measure EAGs. However, when the measuring electrode is placed against the cuticle without penetrating it, good EAGs can be found. Apparently, the resistance of the antenna is low, due to the large internal cavity of the antenna filled with conducting haemolymph. Inserting both electrodes may short-cut the circuit (Den Otter *et al.* 1988). When the cuticle is intact, a potential difference over this higher resistance can be measured. In moths, the haemolymph-filled cavity of the antenna is much smaller, and the resistance from base to tip is about 100 kW (Kaissling, 1971). Therefore, in moths, EAGs can be measured with the electrodes both contacting the haemolymph at the base and at the tip respectively.
Figures 2a and 2b show that an EAG measured with the electrode on the tip of an antenna does not differ in size from an EAG, measured with the electrode near the base of the same antenna. Therefore, the size of an EAG in an antenna of this globular shape does not depend on the number of olfactory receptor neurons between the electrodes, contrary to what is found in the filamentous antennae of moths (Nagai, 1981; Mayer et al., 1984; White, 1990).
When the indifferent electrode was inserted into the tip of the antenna, still an EAG with negative polarity was found (Figure 2c). Would EAGs be formed by the summation of the receptor potentials of many cells, lined up between tip and base of the antenna, the EAG would be expected to be reversed in polarity. Our result shows that wherever the indifferent electrode is inserted, the whole haemolymph in the internal cavity of the antenna is grounded. We propose an other mechanism of EAG-formation in globular, large-volume antennae (Figure 1b): Receptor cells still form dipoles under stimulation because the dendrites depolarize and take up cations from the receptor lymph. Placing an electrode onto the cuticle, contact is made through the pores of the olfactory hairs and a negative potential with respect to the haemolymph in the central antennal cavity is recorded. Therefore, the EAG measured over the cuticle is composed of the receptor potentials of receptor cells near the electrode, as already suggested by Crnjar *et al* (1989). When measuring EAGs one should take care in placing the electrode, because of possible differences in sensitivity of receptive fields in different areas on the antennae.

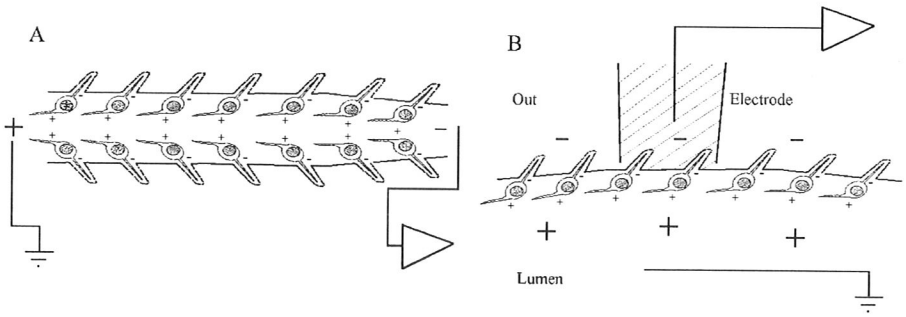

Figure 1. *Upon stimulation, receptor cells become small dipoles because only the dendrite of the cell depolarises. A. In filamentous antennae, EAGs are formed by summation of receptor potentials of many cells from tip to base of the antenna. B. In globular antennae, when the electrode is placed against the cuticle, an EAG is formed by the summated receptor potentials over the cuticula of olfactory receptor neurons near the electrode.*

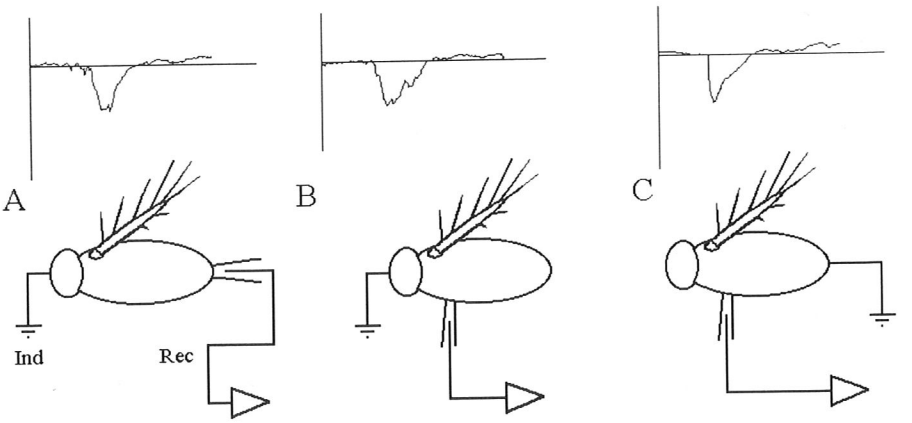

Figure 2. *EAGs obtained by placing the recording (Rec) and indifferent (Ind) electrode at different positions on the antenna, as indicated in the schematic drawing of an antenna below. A. Ind inserted near the base of the antenna and Rec on the tip. B. Ind inserted near the base of the antenna and Rec on the base of the flagellum. C. Ind inserted in the tip and Rec on the base of the flagellum. All three setups give similar EAGs.*

Figure 3 shows the relationship between the size of an EAG and the duration of a pulse of 2 ml 1-octen-3-ol vapour. With a GC it was determined that 35 nanomoles of 1-octen-3-ol were present in this pulse. When the pulse lasted longer, a smaller EAG was found. Therefore, the size of an EAG depends on the steepness of the concentration increase at the antenna. Our amplifier rectifies the baseline, so it was not possible to determine if the area of the EAG is proportional to the dose. If so, the area should remain the same for each pulse duration.
EAG responses to acids sometimes show extreme negative values (Cork and Park, 1996) or EAGs with positive polarity (Warnes and Finlayson, 1986). We tested dose-response series of four acids and found positive EAGs (figure 4). The more acid the stimulus (by acid strenght and dose) the larger the positive potentials.

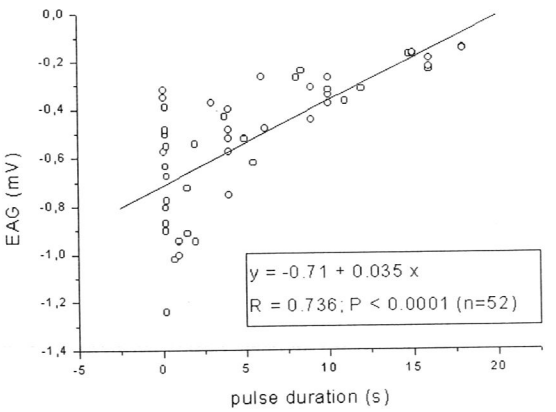

Figure 3. *Electroantennogram responses to pulses of different duration, each containing 35 nanomoles of 1-octen-3-ol in 2 ml air.*

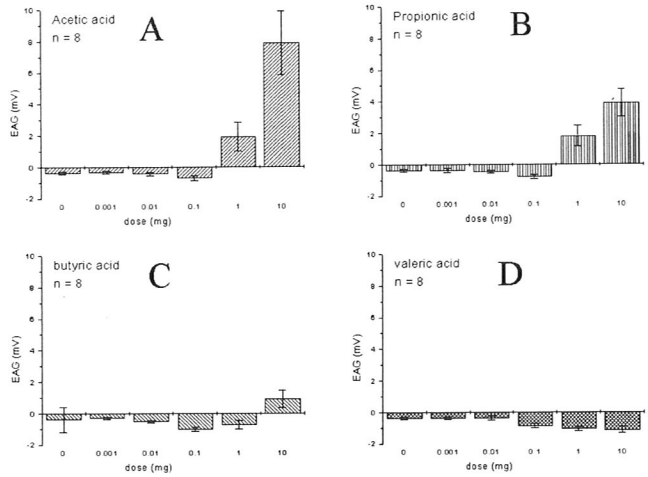

Figure 4. *EAG dose response series to acids with increasing carbon chain length. A. acetic acid; B. propionic acid; C. butyric acid; D. valeric acid.*

After killing the antenna with hexane, no responses to physiological stimuli were found anymore, but positive EAGs to high doses of strong acids still were present (Figure 5). We placed the recording electrode on the rim of the proboscal cavity, where no chemosensory receptor cells are present. Even there positive potentials were measured. This indicates that the positive EAGs in living antennae are no physiological responses. They could be some electrochemical interaction of the electrode with the acid. Single cell responses to these stimuli showed excitation in some cells and inhibition in others. When a majority of cells would be inhibited, a positive EAG would occur. In addition, the positive electrode potential could also inhibit the spike-responses of single cells. Kafka (1970) suggested that inhibition of spike activity by acids is probably due to electrochemical interactions with the electrode material.

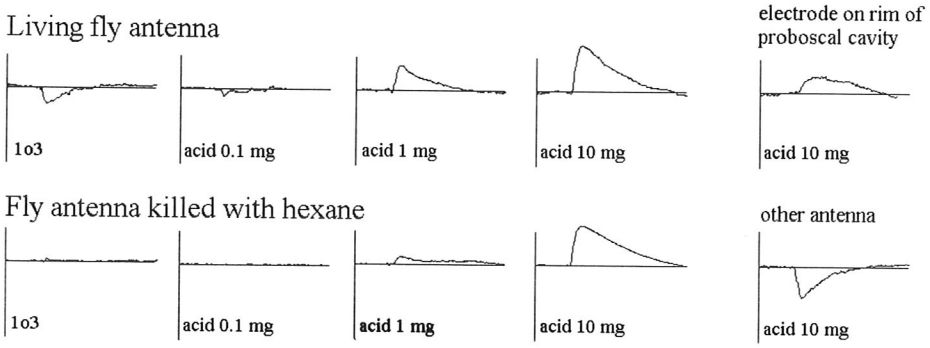

Figure 5. *The upper traces show responses of living flies to 1-octen-3-ol (1o3) and three doses of acetic acid. With the recording electrode on the rim of the proboscis cavity, also a positive potential was measured. Lower traces: after killing the antenna with hexane, no EAGs to 1o3 and 0.1 mg acetic acid were found. Higher doses still evoked positive potentials, and in some other preparations negative potentials were found (last trace).*

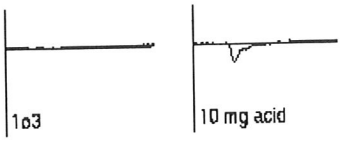

Figure 6. *Replacing the antenna with a filterpaper soaked with Ringer, no responses were found except for stimulation by strong acids that induced negative potentials.*

In different preparations of dead antennae strong acid sometimes evoked negative EAGs (Figure 5). When replacing the biological preparation by a filter paper soaked with Ringer, strong acids induced negative potentials (Figure 6). Meijerink (1999) showed that a cotton thread in contact with tungsten electrodes showed negative electrode potentials when acids were applied, but not when glass electrodes were used. We found electrode potentials with glass electrodes but only at higher doses of acids than used in her study. Probably, ions of the acid change the conductivity of the preparation by diffusing in the Ringer solution in the glass electrode or by hygroscopic activity. Every amplifier has a bias current to be able to measure a potential difference. A change of the resistance changes the potential measured (Van der Pers, pers. comm.). Metal electrodes have large electrochemical potentials and should therefore be avoided for measuring responses to acids. Glass micro pipette/Ag-AgCl electrodes show less artifacts, but responses to strong acids are still artificial. Therefore testing high doses of acids that hardly exist in nature do not show reliable results.

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