Chapter 6

Spatial and temporal characteristics of electroantennograms in flies

Summary

By varying the positions of electrodes on the antenna of houseflies, it was shown that EAGs from globular antennae represent the summated receptor potentials measured over the cuticula of olfactory receptor neurons near the electrode. The size of an EAG depends on the size of the step in odour concentration at the moment the odour strikes the antenna. Physiological responses of olfactory receptor cells to acids can be measured via the EAG at low doses. High doses of strong acids induce electrochemical potentials of the electrodes that vary in strength and polarity, depending on the material of the electrode and the composition of the electrolyte, by the building of a junction potential, or by conductivity changes. 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 for other odorous substances that could be of biological importance for the animal, e.g. indicating food or oviposition substrate. 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, 1991). Each olfactory receptor cell can be considered to become a small dipole under stimulation, because the dendrite of the cell depolarizes most strongly. As a result of the serial arrangement of the sensory cells the receptor potentials of many cells between tip and base of the antenna sum up and the tip of the antenna will become negative with respect to its base (Fig. 1A, after Kaissling, 1971). The shape of the antennae of houseflies and several other Diptera, however, is not filamentous but rather globular and the EAG may be formed in a different way. In this study we investigate what the EAG represents that is measured in housefly antennae on stimulation with odours.

Many insects are attracted by acids and the sensitivity to acids is often investigated using EAG recordings. On stimulation with low doses of acids the EAG is a negative potential, as is found in response to many other types of chemicals. At high acid doses, however, sometimes positive EAGs are recorded (e.g. Warnes and Finlayson, 1986), but also extremely high negative EAGs have been reported (Cork and Park, 1996). As has already been shown by Kafka (1970), on stimulation with acid and alkaline substances slow potentials may occur, which result from electrochemical reactions with the electrodes. Because these potentials may interfere with the physiological responses, we investigated the EAGs obtained in houseflies that were induced by acids in more detail.

Materials and methods

Mature, female houseflies (Musca domestica L.) were used. An intact fly was immobilized in a Finn-pipette with its head protruding out of the tip. EAGs were recorded with glass micropipette / Ag-AgCl electrodes filled with Ringer solution. A standard stimulus was prepared by dissolving 1 mg 1-octen-3-ol in 25 µl silicon oil. To investigate effects of acids, acetic acid, propionic acid, butyric acid and valeric acid were used.

Solutions of 10 mg in 25 µl silicon oil and four decadic dilutions of each acid were made. A solution was pipetted onto a filter paper inside a Pasteur pipette that served as an odour cartridge. Vapour from an odour-loaded Pasteur pipette was blown for 0.2 s into
an airstream flowing over the antennae.

**Results and discussion**

**Spatial characteristics of the EAG**

When a measuring electrode is inserted into the tip of an antenna of a housefly, with the indifferent electrode inserted near the base of the antenna, EAGs can hardly be recorded. However, when the measuring electrode is placed onto the cuticle without penetrating it, clear EAGs can be obtained. Apparently, the internal resistance of the antenna is low, due to the large internal cavity of the antenna filled with conducting haemolymph. Inserting both electrodes may short-circuit the electrodes (Den Otter and Saini, 1985; Den Otter et al., 1988). The cuticle has a high resistance when it is intact. Therefore, when a current flows through the cuticle a clear potential difference over its high resistance can be measured. In moths, the haemolymph-filled cavity of the antenna is very narrow, and the internal resistance from base to tip is about 100 kΩ (Kaissling, 1971). Therefore, in moths, EAGs can be measured with the electrodes both contacting the haemolymph, one at the base and at the other at the tip of the antenna.

**Figure 1.** Upon stimulation, receptor cells become small dipoles because the dendrite of the cell depolarises most strongly. 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.
In the housefly, an EAG, measured with the recording 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, when the indifferent electrode is inserted near the base of the antenna (Figs 2A, B). Therefore, contrary to what is found in the filamentous antennae of moths, the size of an EAG in a globular shaped antenna does not depend on the number of olfactory receptor neurons between the electrodes. When the indifferent electrode is inserted into the tip of the antenna and the measuring electrode is placed near the antennal base, still an EAG with negative polarity is found (Fig. 2C). Would EAGs in globular antennae 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 therefore conclude to another mechanism of EAG-formation in globular, large-volume antennae (Fig. 1B): Receptor cells, when stimulated, still form dipoles because the dendrites depolarize. Placing an electrode onto the cuticle, contact is made through the pores of the olfactory hairs and the negative potential, with respect to the haemolymph in the central antennal cavity is measured. Therefore, the EAG measured over the cuticle is composed of the receptor potentials of receptor cells near the electrode, as was speculated by Crnjar et al. (1989). When measuring EAGs one should take care in placing the electrode, because of possible differences in sensitivity of regionalized receptive fields of the antennae. This phenomenon was found in Drosophila antennae (Ayer and Carlson, 1992).

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

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

Temporal characteristics

Figure 3 shows the relationship between the amplitude of an EAG and the duration of a pulse of 2 ml 1-octen-3-ol vapour, containing 35 nmol of 1-octen-3-ol, as determined by gas chromatography. When the pulse lasted longer, a smaller EAG was found. The amount of odour was the same but diluted in time. As the EAG starts at the beginning of the odour pulse, the size of an EAG depends on the concentration step that arrives at the antenna.

EAGs obtained on stimulation with acids

EAG responses to acids sometimes give extreme negative values (Cork and Park, 1996) or EAGs with positive polarity (Warnes and Finlayson, 1986). On applying dose-response series of the four acids we found positive EAGs at high doses (Fig. 4) except for the application of valeric acid, which always induced negative EAGs. The more acid the stimulus (by acid strength and dose) the larger the positive potentials.

After killing the antenna with hexane, no responses to physiological stimuli are found anymore, but high doses of strong acids still elicit positive potentials (Fig. 5). These potentials even occur when the electrode is placed on the rim of the proboscal cavity, where no chemosensory receptor cells are present. This indicates that these positive EAGs in living antennae are non-physiological responses. Possibly the acid has some electrochemical interaction with the electrode. Strong acid stimuli induce in some olfactory receptor cells a depolarization, resulting in an increase of spike frequency (excitation); but in other cells the opposite occurs (see Chapter 4). When the majority of cells contributing to the EAG would be inhibited, their hyperpolarizations would produce
**Figure 4.** EAG dose response series of antennae to acids with increasing carbon chain length. A) acetic acid; B) propionic acid; C) butyric acid; D) valeric acid.

**Figure 5.** The upper traces show responses of living flies to 1-octen-3-ol (1o3) and three doses of acetic acid. Placing the electrode on the rim of the proboscal 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 evoked still positive potentials, and in some other preparations, negative potentials were found (last trace).
Chapter 6

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

a positive EAG. Alternatively, a positive electrode potential could inhibit the spike-responses of receptor cells. Kafka (1970) suggested that inhibition of spike activity by acids is probably due to potentials resulting from electrochemical interactions of the acids with the electrode material.

In some preparations of dead antennae strong acids induced negative potentials (Fig. 5). When the biological preparation was replaced by a filter paper soaked with Ringer, strong acids induced negative potentials (Fig. 6). Meijerink (1999) showed that the application of acid vapour generates negative electrode potentials between tungsten electrodes, connected by a cotton thread soaked in Ringer, but not between similarly connected glass electrodes. Metal electrodes are easily polarizable, depending on the electrolyte composition, and therefore are less suitable to measure true DC potential differences (Offner, 1967). When the amplifier draws current, electrolysis takes place at the interface between metal and solution and an electrochemical potential builds up. Different ions will contribute to the electrode reactions, depending on the electrode potential. The electrode potential of tungsten electrodes depends on the pH of the electrolyte (Frank and Becker, 1964), and thus artifacts may be found when using tungsten electrodes to measure responses to acids.

To avoid metal electrode polarization, we used non-polarizable Ag/AgCl electrodes in Ringer-filled glass micropipettes. When current flows between these electrodes, electrolysis will not change the direct environment of the electrode, as AgCl will be reduced to metallic silver, freeing the chloride ions into the solution, while Cl⁻ from the electrolyte solution will be deposited onto the anode, increasing the amount of AgCl on it. Still, we do find acid-induced electrode potentials with glass micropipette Ag/AgCl electrodes, but only at higher doses than used by Meijerink (1999). This can be explained by the building of a junction potential at the interface of the electrolyte solution in the micropipette and the solution on the antennal surface or the cotton thread, in which acid from the stimulation puff dissolves. This junction potential ($E_j$) or diffusion potential is described by a modified Nernst equation:
where \( R \) is the gas constant, \( T \) is the temperature, \( n \) is the valency of the ion (1 for acids), \( F \) is Faraday’s constant; \( u \) is the diffusional mobility of the cation (H\(^+\)), \( v \) is the mobility of the anion (R-COO\(^-\)) and \( c_1 \) and \( c_2 \) are the concentrations on the opposite sides of the interface. The mobility of the H\(^+\) cation (3.63\( \times \)10\(^{-7} \) m\(^2\)/s/V) is much larger than the mobility of the anions and dominates the current flow. The concentration \( c_1 \) inside the micropipette is low (10\(^{-7} \) M at pH = 7) compared to \( c_2 \) and thus a negative junction potential will be formed. This is seen in Fig. 6. However, we also found positive potentials upon stimulating the antennae with acids. Probably ions of the acid change the conductivity of the preparation by diffusing in the sensillum lymph or in the Ringer solution of the glass electrode or by hygroscopic activity (Van der Pers, pers. comm.). A change of the resistance thus can result in a potential change.

Metal electrodes have large electrochemical potentials and should therefore be avoided when measuring responses to acids. Glass micropipette/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 does not produce useful information about the sensory physiology of the olfactory system.

References


