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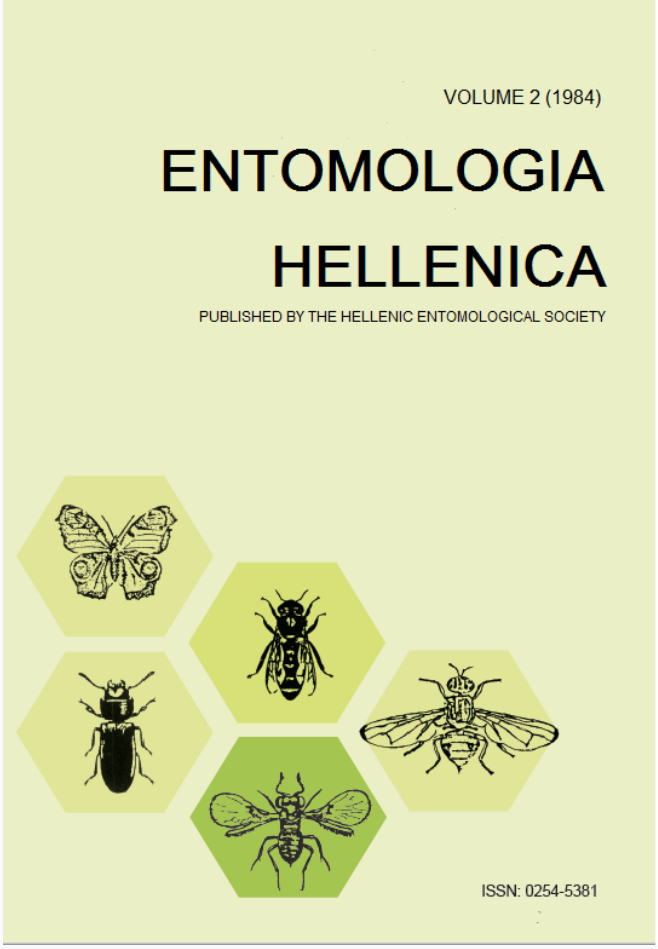

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Electroantennogram Responses from Olfactory Receptors in *Dacus oleae* [1]

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ABSTRACT

Substances found in *Dacus oleae* (Gmelin) (Diptera: Tephritidae) male or female flies which have been reported as pheromones, i.e. elicit some form of biological activity in laboratory or field bioassays, were tested with the electroantennogram technique (EAG). Substances of non-insect origin were also tested as possible pheromone candidates. All substances of insect or non-insect origin elicited an EAG response to both sexes of lab-cultured or wild insects but 1,7 dioxaspiro [5,5] undecane, the major pheromone component, has a lower response threshold value than all other compounds. At the maximum stimulus concentration the response to nonanal reaches a value higher than that elicited by all other compounds. EAG responses to various compounds, after receptor adaptation to the major pheromone compound and nonanal, showed that these two compounds are detected by different sets of receptors. Other comments on the sensitivity and specificity of antennal receptors are also presented.

Introduction

Dacus oleae (Gmelin) (Diptera: Tephritidae) relies heavily on chemical stimuli for communication with members of the same species and for monitoring its environment. Several aspects of the behavior of the insect have been found to be regulated by chemical signals.

Females release an airbore sex pheromone which functions as a strong, long-range male attractant (Haniotakis 1974, 1977, Haniotakis et al. 1977). This pheromone was found to be a blend of the following four substances: 1,7-dioxaspiro (5.5) undecane; α-pinene; nonanal and ethyl dodecanoate (Baker et al. 1980, Mazomenos and Haniotakis 1981, Mazomenos et al. 1981). 1,7-dioxaspyro (5.5) undecane is the major component of the blend on the basis of both quantity and biological activity. Gariboldi et al. (1982) isolated the substances E-6 nonen-l-ol and p-cymene from female *D. oleae* flies which, they claim, displayed attractive and aphrodisiac effects in both laboratory and field tests.

The major component of the female pheromone blend as well as the compound 5-oxo-1,9-nonadioic acid, diethyl ester, were found in the rectal glands of wild *D. oleae* male flies. Both substances were attractive to males in laboratory bioassays (Mazomenos and Pomonis 1983). The role of these substances is not known. Tests with antennectomized females have shown that chemical stimuli perceived by female antennal receptors are required for successful mating. An arrestant effect of the major female pheromone component to the female flies has also been conjectured (Haniotakis et al. in press). De Marzo et al. (1978) reported that *D. oleae* males strongly attract females by a sex pheromone, at the time of day when the species is sexually active. Haniotakis (1974, 1977) failed to show long-range female attraction by

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males both in laboratory and field experiments. The presence of pheromones in *D. oleae* flies has also been conjectured on the basis of anatomical studies or odors as perceived by humans (Economopoulos et al. 1971, Schultz and Boush 1971).

Volatile substances emitted by olive fruit at a certain physiological state act as attractants for ovipositing females which guide them to locate suitable oviposition substrata (Fiestas Ros de Ursinos et al. 1972). Volatile substances present in the olive fruit act as oviposition deterrents which divert females from ovipositing in already used fruit. These substances are water soluble (Cirio 1971) or liposoluble (Girolami et al. 1981b). Haniotakis and Voyadjoglou (1978) have shown that physical characters of the olive fruit in combination with chemical stimuli regulate oviposition in *D. oleae*. Volatile substances obtained by methanol extraction of olive fruit act as oviposition stimulants for *D. oleae*. This activity is due to at least two substances, which during the summer may also act as attractants (Girolami et al. 1981α). It is possible that the oviposition attractants reported by Fiestas Ros de Ursinos are the same as the oviposition stimulants found by Girolami et al. Fletcher et al. (1978) have found that a stimulus from the olive fruit, probably nutritional, interacting with environmental temperature and humidity, determines ovarian maturation in *D. oleae* females during the summer months.

Strong indications exist that the dispersal of *D. oleae* during the summer and its concentration in olive orchards with fruit receptive to attack is based on chemical signals (Lupo 1943, Girolami 1978, Fletcher 1979). Chemical and physical characters of the olive leaves or branches were found to be essential for host recognition in this species while females locate individual fruits by visual characters, in particular shape, color and size, while after arriving on the fruit chemo- or physico-tactile cues enable them to distinguish suitable oviposition sites (Prokopy and Haniotakis 1976, Prokopy et al. 1975).

Experiments with antennectomized insects showed that odor perception in both male and female *D. oleae* flies is achieved by sensory structures located on the antennae (Haniotakis unpublished data). The morphology and fine structure of these chemoreceptors were studied by Hallberg et al. (1984).

In the present study the electroantennogram (EAG) technique was used as a bioassay to determine the responses of *D. oleae* to pheromone components and possible pheromone candidates.

Materials and Methods

Olive flies were obtained from a culture maintained on artificial diets at "Democtitos" N.R.C. for 30 generations, as well as from wild populations. After emergence, flies were kept in screen cages under conditions described by Mazomenos and Haniotakis (1981).

In preparation for EAG recordings the head of an insect was excised and attached to an electrolyte-filled glass capillary electrode. The electrolyte consisted of a 0.1 M solution of KCl containing 10% by volume of polyvinylpyrrolidone K90 (Fluka AG, Buchs, Switzerland). The tip of the electrode extended through the head as close as possible to the pedicellus of one of the antennae. The tip of a similar electrode was brought into contact with the distal end of the funiculus. The preparation was connected via Ag-AgCl wires in the glass capillary electrodes to the input of a high impedance DC amplifier. The output signal of the amplifier was displayed on the screen of a storage oscilloscope. EAG responses were measured from the oscilloscope screen.

The odor delivery system and stimulation technique were essentially the same as that described by Van Der Pers (1981). A constant flow (0.5 m/s) of charcoal filtered air was passed over the antenna through a glass tube (i.d. 7 mm), the end of which came within 5 mm from the preparation. Stimulation was achieved by mixing 1 ml of the contents of a disposable 5 ml plastic syringe into the constant air stream during 0.1 s by means of a spring-activated injection device (Murphy Developments, Hilversum, The Netherlands). The syringes contained a 2 cm^2 piece of filter paper onto which the test compound was applied in amounts ranging from 10^{-1} to 10^{4} μg dissolved in 25 μl paraffin oil. Test compounds, including sources and indicated purities, are listed in Table 1. All chemicals of male or female origin which have been reported as pheromones, i.e. elicit some form of biological activity in laboratory or field bioassays, are included in this table except for E-6-nonen-1-ol, which was not available at the time. Substances of non-insect origin which were tested were selected for specific reasons, e.g. 4-chloro-1-butanol is a substance produced in an intermediate step of the synthesis of 1,7-dioxaspiro (5.5) undecane; 2-butyl-4 methyl-1,3-dioxane has a stucture similar to that of the major pheromone component (1,7-dioxaspiro [5.5] undecane); β-pinene was tested in comparison to α-pinene; 1-octanol and 1-decanol were tested instead of the unavailable E-6-nonen-1-ol.

TABLE 1. List of test compounds, purity, and sources.

Nr. Compound	% purity	source	origin
1. 1,7-dioxaspiro (5.5) undecane	99.5	Vioryl S.A. (Athens, Greece)	F, M*
2. α-pinene	95	Fluka AG (Switzerland)	F
3. β-pinene	95	Fluka AG	N
4. 5-oxo-1,9-nonadioic acid, diethyl ester	95	Fluka AG	M
5. Ethyl dodecanoate	99	Vioryl S.A.	F
6. 4-chloro-1-butanol	95	Fluka AG	N
7. p-cymene	98	Vioryl S.A.	F
8. 2-butyl-4-methyl-1,3-dioxane	95	Vioryl S.A.	N
9. nonanal	99	Vioryl S.A.	F
10. 1-octanol	99	Vioryl S.A.	N
11. 1-decanol	99	Vioryl S.A.	N

* F = of female insect origin, M = of male insect origin, N = non-insect origin.

Two sets of experiments were performed. In the first set different concentrations of test substances were mixed into a pure constant air stream blown over the preparation and the resulting EAG's were recorded. In the second set, the constant air stream was charged with a relatively high concentration of one of the test compounds (introduction of a loaded filter paper into stream line) and the EAG's were measured during additional stimulation with each of the remaining compounds. No correction was made for differences in volatility among the test compounds. Comparison between the responses elicited are therefore only relative.

Results

The EAG responses evoked during stimulation with equal concentrations of the various test compounds are shown in Fig. 1, for both male and female wild and laboratory-cultured olive flies. All compounds elicit an EAG response higher than that evoked by the solvent, paraffin oil. The latter response may be attributed to activation of mechanosensitive sensilla due to the slight fluctuation in air speed during stimulation. Differences between the response spectra of males and females and of wild and laboratory-cultured insects are not significant within the limits of standard error.

On the average, the highest responses were obtained by stimulation with nonanal and 1-octanol followed by 4-chloro-1-butanol. Intermediate responses were evoked by α-pinene, β-pinene, p-cymene, 2-butyl-4-methyl-1,3-dioxane and 1-decanol. Responses only slightly higher than those to the solvent were evoked by 5-oxo-1,9-nonadioic acid diethyl ester and ethyl dodecanoate.

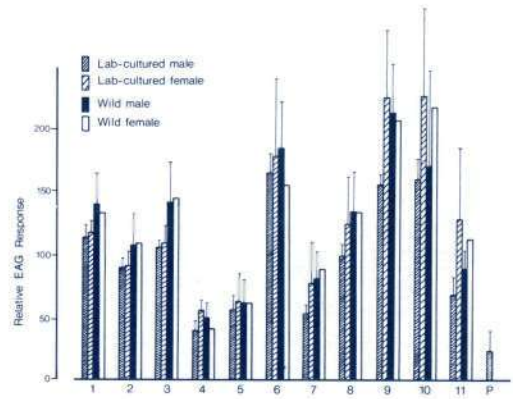

FIG 1. Relative EAG responses from lab-cultured and wild male and female *D. oleae* antennae during stimulation with 11 different substances at a concentration of 10^3 µg on the odor sources. The substances are numbered 1 to 11 corresponding to the compounds given in Table 1. Vertical lines on top of bars indicate upper half of standard error. The experiments were replicated three times, except for the measurements on the wild female. P = response of lab-cultured males and females to the solvent paraffin oil, 1 mV = 50 of the ordinate scale.

Dose-response curves for the main pheromone component (1,7-dioxaspiro (5.5) undecane), nonanal, α-pinene, ethyl dodecanoate and 5-oxo-1,9-nonadioic acid diethyl ester are presented in Fig. 2. At the maximum stimulus concentration of 10^3µg the response to nonanal reaches a value higher than

that elicited by the main pheromone component. For all concentrations below 10^3 µg the highest responses are measured to the main pheromone component. At concentrations of 10^{-1} and 1 µg only the main pheromone component evokes EAG's higher than the EAG elicited by application of the solvent, paraffin oil. This indicates that the response threshold value for the main pheromone component is much lower than that of the other compounds tested.

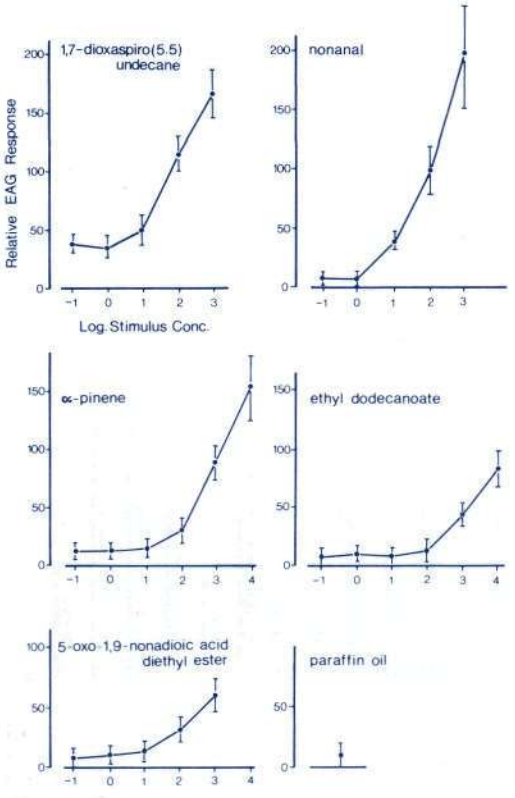

FIG. 2. Dose-response curves for five substances measured during stimulation with increasing concentrations. EAG response scale and concentration scale (in µg) refer to all curves. Vertical lines associated with each point indicate standard error (N = 3). 1mV = 50 of the ordinate scale.

Representative EAG recordings for the test compounds at a concentration of 10^3 µg, are presented in Fig. 3. It can be observed that, with the exception of 5-oxo- 1,9-nonadioic acid diethyl ester, the shapes of the various responses do not differ significantly. The EAG response to 5-oxo-1,9-nonadioic acid diethyl

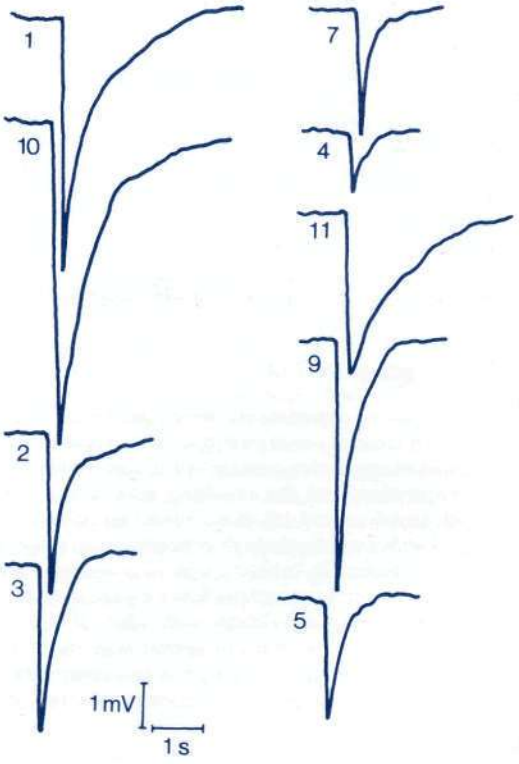

FIG. 3. Typical examples of EAG recordings. The numbers refer to the compounds listed in Table 1. Horizontal and vertical scale refers to all recordings.

ester shows a slower recovery after maximum depolarization than responses to the remaining test stimuli.

The EAG responses obtained from the test compounds after the constant air stream was charged with a high concentration of either the main pheromone component or nonanal are shown in Fig. 4. The figure shows that during continuous stimulation with the main pheromone component (Fig. 4a) the responses to the remaining test compounds do not disappear completely. In particular, the responses to ethyl dodecanoate, nonanal, 1-octanol and 1-decanol are still relatively high. The responses to the main pheromone component are very low in this experiment, indicating that the sensory cells responding to this compound were almost com-

pletely adapted.

Continuous stimulation with nonanal (Fig. 4b) shows that an EAG was evoked by the main pheromone component and weak responses by β-pinene and 1-octanol. The response to additive stimulation with nonanal itself was not different from that to air. This implies that the receptors responding to nonanal were completely adapted to the continuous stimulation. The response to the main pheromone component is obviously evoked by other receptors not adapted by the continuous nonanal stimulation.

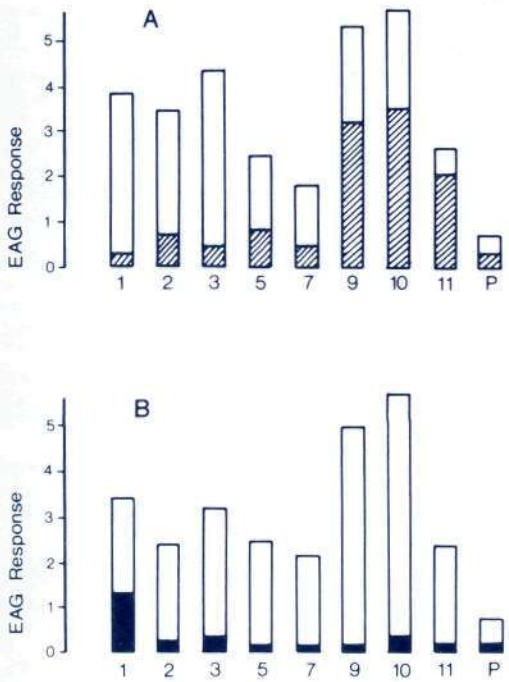

FIG. 4. EAG responses obtained during stimulation with 8 compounds (numbered 1-11, Table 1) and paraffin oil (P) alone (open bars) at a concentration of 10^3 µg; additional stimulus after saturation of the antenna by continuous stimulation with the main pheromone component (A) or nonanal (B) at a concentration of 10^3 µg. Hatched and black areas show response obtained by additional stimulus. EAG response in mV.

Discussion

Considering the importance of chemical communication in *D. oleae*, both with respect to reproductive behavior and host-plant relationship, the presence of a sensitive and probably selective olfactory receptor system may be expected in this species. Such a system has been found in different species of diptera (Guerin and Städler 1982), but seems to be absent in several other monophagous insects (Visser 1979).

Our results show that antennal olfactory receptors of male and female *D. oleae* adults are sensitive to 1,7-dioxaspiro (5.5) undecane, α-pinene, nonanal and ethyl dodecanoate, the four compounds constituting the female sex pheromone (Baker et al. 1980, Mazomenos and Haniotakis 1981, Mazomenos et al. 1981) as well as to p-cymene. The main pheromone component, which constitutes more than 50% of the female effluvium (Mazomenos and Haniotakis 1981), however, does not elicit the highest EAG response. Among the female pheromone components the highest EAG response is evoked by nonanal. Nonanal is present in the female effluvium in amounts about ten times lower that the main pheromone component (Mazomenos and Haniotakis 1981). Studies in moths have shown that there is not always a positive correlation between the male EAG's and the relative amounts of the female pheromone components (Van Der Pers and Löfstedt in press). The high EAG response to nonanal may be explained by assuming that either a large number of receptor cells respond to this compound, or that the receptor cells have a higher sensitivity for nonanal. By examining the lower stimulus intensities in the dose-response curves (Fig. 2), however, the latter explanation can be excluded: responses to the main pheromone component are higher than those to nonanal.

The fact that compounds of non-insect origin (e.g. β-pinene, 2-butyl-4-methyl-1,3 dioxane, octanol and decanol) elicit EAG responses of similar or even higher magnitudes than compounds of insect origin may indicate that the receptors activated by these stimuli are not very selective. Moreover, the shape of the EAG responses, which may be typical for a certain stimulus, does not show significant variations among the stimuli tested (Fig. 3). From standard EAG measurements it is not possible to draw definite conclusions about selectivity. For this reason we conducted two EAG experiments in which the receptors were adapted to either the main pheromone component or nonanal prior to stimulation with other compounds.

During continuous stimulation with the main pheromone component, subsequent additional stimulation with this compound did not elicit a significant additional response (Fig. 4a). Thus the receptors involved were completely adapted. Additional stimulation with nonanal, octanol and decanol, on the other hand, resulted in relatively high EAG responses, albeit lower than those evoked during stimulation with these compounds alone. This result shows that at least two different types of receptors are present: one type, activated by the main pheromone component and α-pinene, β-pinene, ethyl dodecanoate and p-cymene, and another type of cells responding mainly to nonanal, octanol and decanol. This conclusion is supported further by the result of a complementary experiment in which the receptors were continuously stimulated by nonanal prior to additional stimulation with the other compounds. In this experiment (Fig. 4b) only the main pheromone component gave a reasonable EAG response during additional stimulation. It is possible that the antennae of *D. oleae* contain receptor cells exclusively tuned to the main pheromone component in addition to receptor cells responding to a large variation of substances.

From the EAG experiments presented it is clear, however, that many of the olfactory receptors on both male and female *D. oleae* flies are not specifically tuned to one compound only. There seems to be a high degree of overlap in sensitivity spectra of these receptors. A large variety of olfactory stimuli play a part in the behavior of *D. oleae*. Antennal receptors may be more or less tuned to the perception of some specific odors. Other odors may be discriminated by the insect based on the combined responses of many different types of receptor cells.

Hallberg et al. (1984) describe the morphological characteristics of three different types of sensilla present on the surface of the funiculus of the *D. oleae* antennae and two types located in the olfactory pits. On morphological grounds, these sensilla are assigned an olfactory function. Differences in distribution or morphlogy of these receptors between males and females were not found. This fact is reflected in the similarity between male and female *D. oleae* EAG responses reported in the present study. It is likely that males and females of this species are equally well able to perceive a similar set of olfactory signals, including male and female sex pheromones.

More information about the specificity of the receptor cells on the antennae of *D. oleae* can only be obtained by analysis of recordings from single olfactory sensilla.

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KEY WORDS: *Dacus oleae*, Olive fruit fly, Diptera, Tephritidae, Electroantennogram, Electrophysiology, Pheromones

Ηλεκτροαντενογραφικές Αντιδράσεις των Αισθητηρίων Οσφρήσεως του *Dacus oleae*

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ΠΕΡΙΛΗΨΗ

Ουσίες που βρέθηκαν σε αρσενικά ή θηλυκά άτομα του εντόμου *Dacus oleae* και που αναφέρονται σαν φερομόνες, διεγείρουν δηλαδή κάποια μορφή βιολογικής αντίδρασης σε δοκιμές εργαστηρίου ή αγρού, εξετάστηκαν με την τεχνική του ηλεκτροαντενογράφου (ΗΑΓ). Ουσίες που δεν απαντώνται στο έντομο αυτό και που επελέγησαν για ειδικούς λόγους, εξετάστηκαν επίσης με την ίδια τεχνική σαν πιθανές φερομόνες. Όλες οι ουσίες που εξετάστηκαν ανεξάρτητα αν απαντώνται στο έντομο ή όχι, έδειξαν αντίδραση κάποιου βαθμού και στα δυο φύλα του εντόμου που προέρχονταν είτε από τεχνητή εκτροφή είτε από άγριους πληθυσμούς. Η ουσία 1,7-διοξασπυρο (5,5) ενδεκάνιο, η κύρια φερομόνη του δάκου, έδειξε την ισχυρότερη αντίδραση σε μικρές συγκεντρώσεις. Στη μεγαλύτερη συγκέντρωση που δοκιμάστηκε, η εννεανάλη, ένα από τα τέσσερα συστατικά του μίγματος φερομόνης, έδειξε την ισχυρότερη αντίδραση. Οι αντιδράσεις του εντόμου στις διάφορες ουσίες μετά από εθισμό των αισθητηρίων οσφρήσεως στην κύρια φερομόνη ή στην εννεανάλη, έδειξαν ότι οι δυο αυτές ουσίες ανιχνεύονται από διαφορετικά αισθητήρια. Η ευαισθησία και η εξειδίκευση των αισθητηρίων σχολιάζεται με βάση τις ηλεκτροφυσιολογικές αυτές παρατηρήσεις.