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Neurophysiological and behavioural evidence for an olfactory function for the dorsal organ and a gustatory one for the terminal organ in *Drosophila melanogaster* larvae

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Abstract

Multicellular electrophysiological responses from the dorsal organ on the cephalic lobes of third instar *Drosophila melanogaster* larvae (wild-type Canton S) stimulated with a cold-trapped banana volatile extract showed that this structure has an olfactory function in the fruit fly. Responses of the dorsal organ were also recorded to constituents of the banana volatile extract as they eluted from a gas chromatographic column (GC-coupled dorsal organ electrophysiology). The active chemostimulants were identified as 2-heptanone, isoamyl alcohol, hexyl acetate, hexanol and hexyl butyrate by gas chromatography-coupled mass spectrometry. Applying the same recording system to the terminal organ sensilla, no responses were obtained to either the banana volatile bouquet or its constituents. By contrast, high frequency multicellular responses were recorded in response to touching the terminal organ with the gustatory stimuli KCl and grapefruit juice; responses were absent on similar stimulation of the dorsal organ with either NaCl or KCl. This suggests a role for olfaction by the dorsal organ and for gustation by the terminal organ in *Drosophila* larvae.

In a 7-mm high wind tunnel with a thin 1.2% agar floor, the *Drosophila* larvae showed odour-conditioned upwind responses in an air stream of 0.1 m/s bearing banana volatiles. *Drosophila* larvae responded best to the odour of cut bananas. A 1:1 mixture of the banana odour constituents 2-heptanone and hexanol (at either 50 or 100 μg source dose each) proved as attractive as the known larval attractants propionic acid and isoamyl acetate on their own at 100 μg, whereas hexanol and 2-heptanone on their own at a 100 μg source dose were less attractive. The stronger behavioural response to the banana volatile bouquet and to the binary mixture serves to underline the multireceptor nature of the dorsal organ response to food odour in *Drosophila*.

Keywords: *Drosophila*; Neurophysiology; Behaviour; Olfaction; Gustation

1. Introduction

The third antennal segment of *Drosophila*, the funiculus, bears about 500 sensilla belonging to three morphological types, basiconic, trichoid and coeloconic (Riesgo-Escovar et al., 1997a). Recordings from single basiconic sensilla have shown that the receptors they house respond to volatiles such as ethyl acetate, isoamyl acetate, butanol, cyclohexanone, benzaldehyde (Siddiqi, 1991) and in electroantennogram recordings (EAG, Schneider, 1957) the antennae sensilla have shown responses to esters such as ethyl acetate and alcohols such as butanol (Venard and Stocker, 1991). Scanning electron micrographs have revealed the presence of pores on both the basiconic and trichoid sensilla (Riesgo-Escovar et al., 1997a), typical for an olfactory function (Altner, 1977). The maxillary palps constitute a second pair of olfactory organs in *Drosophila*, bearing about 60 basiconic and about 20 trichoid sensilla each (Riesgo-Escovar et al., 1997b), and electropalpograms (EPG) have been recorded in response to stimulants such as ethyl acetate, butanol and benzaldehyde (Riesgo-Escovar et al., 1995). Selective ablation of either antennae or palps has shown that each contributes to the behavioural response of flies to volatiles (Charro and Alcorta, 1994). In addition, the two olfactory organs have a common origin in the eye-antenna imaginal disc, as opposed to

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the taste bristles of the labellum which develop from the labial discs (Ray et al., 1993).

The larvae of *Drosophila* possess three pairs of prominent cephalic sensory organs: dorsal, terminal and ventral (Fig. 1; Singh and Singh, 1984). Ultrastructure studies indicate that only the central dome (Fig. 1) of the dorsal organ is suggested to have an olfactory function in *Drosophila* as in other Diptera such as *Musca* (Chu and Axtell, 1971) and *Hylemya* (Honda and Ishikawa, 1987a), as the cuticle is perforated and the sensory dendrites are in contact with the exterior through a pore–tubule system. Peripheral receptors of the dorsal organ, and of the entire terminal and ventral organs, are suggested from ultrastructure studies to have gustatory and mechanosensory functions in *Drosophila* (Singh and Singh, 1984), *Musca* (Chu and Axtell, 1971; Chu-Wang and Axtell, 1972a,b) and *Hylemya* (Honda and Ishikawa, 1987a). Here, the dendrites communicate with the exterior through a single opening and incorporate a tubular body in certain cases.

Although the olfactory organs have a common function in adults and larvae, they have different developmental origins. During metamorphosis, the larval dorsal organ is histolysed (Stocker, 1994), and the antennae and palps develop de novo from the eye-antenna imaginal disc (Postlethwait and Schneiderman, 1971). Whereas the larval dorsal organ dome houses dendrites from 21 sensory cells, the antenna bears some 1200 sensory cells. The simplicity of the larval olfactory system is also reflected in the apparent absence of major sites of convergence and integration in the brain such as glomeruli, structures which are well developed in the adults. Despite this, larvae show a high capacity for chemosensory discrimination, capable of responding selectively to certain volatiles (Ayyub et al., 1990) and to gustatory stimuli (Miyakawa, 1982; Lilly and Carlson, 1990).

Recent developments in molecular genetics, and the increasing number of olfactory mutants which have been isolated, have given new impetus to olfactory studies in *Drosophila*, permitting the identification of certain principles governing its organization and function (Carlson, 1996). Some mutants show morphological deficiencies, such as those on the *lozenge* gene implicated in the development of the antennae and palps. These mutants show a complete absence of basiconic sensilla on the antenna (Riesgo-Escovar et al., 1997a), along with a reduction in size and absence of pores on the basiconic sensilla on the palps (Riesgo-Escovar et al., 1997b). Electrophysiological responses from the palps and antennae, as well as behavioural responses to olfactory stimulants in *lozenge* mutants are strongly reduced compared to wild-type flies (Venard and Stocker, 1991; Riesgo-Escovar et al., 1997a,b). On the other hand, mutations affecting the *olf* gene complex, known to affect both larvae and adults, are suspected to have consequences at the receptor level or on the transduction pathways responsible for the perception of a particular stimulus (Carlson, 1996). Such mutants show deficiencies for the perception of a given class of product such as aliphatic and aromatic aldehydes or acetates, whereas others manifest more pervasive anosmias, and all show shifts in sensitivity to the test products (Siddiqi, 1991).

Despite great gains in our understanding of the functioning of such a complex sensory system as olfaction in *Drosophila*, certain fundamental questions remain unanswered such as the specific roles of the relatively simple cephalic sensory organs of the larvae. This paper describes the electrophysiological responses of the dorsal organ to volatile chemostimuli identified in banana odour, and the behavioural responses of the larvae to these volatiles. Evidence is also provided here for a role of the terminal organ in gustation.

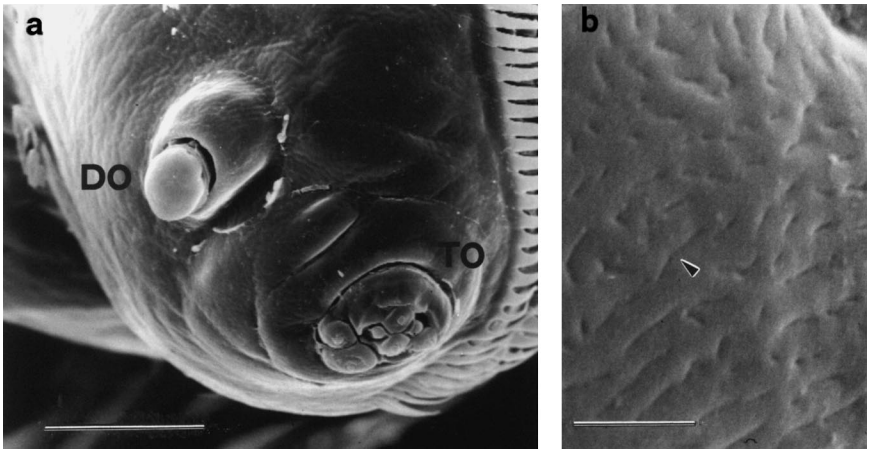

Fig. 1. (a) Anterolateral view of the left cephalic lobe of a third instar *Drosophila melanogaster* larva shows the dorsal organ (DO) and the terminal organ (TO). To record olfactory responses, the recording electrode was inserted in the rim of the dorsal organ, i.e., situated between the dome and the cylindrical supporting portion, and arbitrarily in the terminal organ. To record gustatory responses from the terminal or dorsal organ, the wide-tip-opening electrode covered the sensory structures entirely. (b) Detail of the dome of the dorsal organ shows pores (arrow). The scale bar in (a) is 20 μm and in (b) 500 nm.

2. Materials and methods

2.1. Drosophila rearing

The Canton S strain was obtained from R. Stocker, University of Fribourg, Switzerland. Cultures were grown in 165 ml standard plastic vials with cotton stoppers on a medium containing cornmeal, agar, sugar and dry yeast. When the freshly made medium had solidified, fresh yeast was added. The culture was maintained at 25°C, 60% RH and a 12 h:12 h light:dark cycle in an incubator. Under these rearing conditions, the third instar lasted 48 h (Graf et al., 1992).

2.2. Scanning electron microscopy

Drosophila third instar larvae were fixed in 70% ethanol, dehydrated in 90% ethanol (10 min) and 100% ethanol (3 times 10 min), and critical point dried in CO_2 with a BAL-TEC (Liechtenstein) CPD 030 device. The mounted animals were sputtered with gold in a sputtering apparatus (SCD 005, BAL-TEC) and then observed under a scanning electron microscope (XL20, Philips, The Netherlands).

2.3. Chemicals

A series of 46 volatile synthetics were tested in electrophysiology and some of them also in the behavioural tests: straight chain and branched acetates (C_4–C_{10} obtained from Fluka (Switzerland), Aldrich (Germany), Sigma (Germany), Merck (Germany) and Interchim (France); all were >95% GC), hexyl butyrate (Interchim; 98% GC), straight chain and branched alcohols (C_3–C_8, Fluka; all >95% GC), 2-propyl phenol (Aldrich (USA); 98% GC), aliphatic and aromatic aldehydes (C_2–C_8, Fluka, Merck, Bedoukian (USA) and Oril (France); all >97% GC except (*cis*-3) hexenal 50%), aliphatic and aromatic ketones (C_4–C_7, Fluka; all >98% GC), carboxylic and aromatic acids (C_3–C_8, Fluka and Merck; all >98% GC), monoterpenes (ß-myrcene, Sigma, 90% GC and (+)-α-pinene, Fluka, >99% GC), straight chain and branched monoamines (C_5–C_7, Fluka and Sigma; all >97% GC) and ammonia 25% (Fluka, >97% GC). Hexane and dichloromethane, both analytical grade, and KCl and NaCl, both >99% pure, were from Merck; glucose, >99% pure, was from Fluka.

2.4. Banana odour extracts

Two methods were used to collect banana odour. For a cold-trapped extract, about 300 g of chopped banana (pulp and skin) were put in a 500-ml gas-wash-flask. A 58-cm glass U tube (4 mm i.d.) was placed into a Dewar flask filled with acetone and dry ice (−80°C), and charcoal-filtered air was drawn at 100 ml/min over the banana into the U tube. After 60 min, the condensate was mixed thoroughly with 1 ml dichloromethane and the organic fraction was separated into 50 µl aliquots for storage at −80°C. Banana volatiles were also collected on Porapak Q® to increase the yield of products collected. Porapak® (50–80 mesh, Milipore Corporation, USA), a porous polymer which selectively desorbs water while retaining a large spectrum of volatiles was conditioned by extraction with CH_2Cl_2 in a Soxhlet extractor for 12 h, and by drying under N_2 (200 ml/min) at 180°C for 30 min. About 500 mg of conditioned Porapak® was packed into the barrel of a Pasteur pipette (70 mm long, 5 mm i.d.) with glass wool stoppers at each end, and this cartridge was connected to a 1-l gas-wash-flask containing about 600 g of chopped banana (pulp and skin) held at 38°C in a water bath. Charcoal-filtered air was drawn at 100 ml/min over the banana for 30 min onto the Porapak® filter. Volatiles were eluted from the polymer with CH_2Cl_2 and stored at −80°C in 50 µl aliquots.

2.5. Electrophysiology

Larvae used in electrophysiology were taken from the culture during the first 24 h of the third instar (48–72 h after egg hatching), as the cuticle of these individuals was found to be less resistant to penetration by the recording electrode (below). The larva was placed on its dorsum on a match stick and a silver wire placed beneath it served as a reference electrode. The head and body were rendered immobile using dental floss as a ligature. The mounted larva was placed under a Wild M3Z Kombistereo microscope (Leica, Switzerland; magnification of 500, working distance 11 mm).

For recording olfactory responses from the cephalic sensory organs a drawn out glass capillary (1.5 mm o.d., 0.87 mm i.d.) with a 1-µm closed tip and back-filled with 0.05% polyvinylpyrrolidon K90 (Fluka) in Kaissling SLR (sensillum lymph ringer; Kaissling and Thorson, 1980) was used as the recording electrode. This was inserted at the rim of the dome of the dorsal organ or in the terminal organ, using a micromanipulator (Leitz, Germany), whereby the tip of the electrode broke on entry. The recording electrode was connected via a chlorinated silver wire (0.38 mm in diameter) to a high impedance preamplifier (Syntech, The Netherlands), mounted on the micromanipulator, and thence to a universal AC/DC amplifier (UN-03, Syntech, The Netherlands). Signals were amplified 1000×, visualized on an oscilloscope (Tektronix, USA), and recorded via an analog-digital interface in an IBM compatible PC equipped with the spike analysis software Autospike (Syntech, The Netherlands).

Charcoal-filtered air (25°C and 90% RH) was blown continuously over the preparation at 1 m/s from a 6-mm i.d. glass tube whose orifice was 5 mm from the larva. This water-jacketed tube, circulating water from a water

bath, served to maintain constant conditions in the airflow. The needle of a 5-ml plastic syringe containing the odour (synthetic chemicals, fresh banana or banana extract) was introduced through a septum-covered hole in the glass tube bearing the air-stream, 22 cm from its outlet. A charcoal-filtered air pulse, delivered by a solenoid valve from a stimulator (CS-02S, Syntech, The Netherlands), was administrated via a stopper at the plunger end of the syringe, so that 1 ml of the syringe content was injected in 1 s into the glass tube. To prevent changes in the air flow during stimulation, a charcoal-filtered airflow of 1 ml/s was delivered via a second solenoid valve through a blank syringe into the air-stream at the same distance from the preparation during stimulus off.

To record gustatory responses from the cephalic sensory organs, some points of the electrophysiology setup described above were modified. First, the tip of the recording electrode was cut to provide a 12–15 μm tip opening. This covered the dorsal or terminal organ entirely. No effort was made to record from selected sensory structures of the terminal organ due to their small size (Fig. 1). The recording electrode contained either Kaissling SLR, 100 mM KCl, 100 mM NaCl, 100 mM glucose+100 mM NaCl, or commercial grapefruit juice concentrate (diluted approximately 10 times) in 100 mM NaCl. The reference electrode was a drawn out glass capillary (2 mm o.d., 1.08 mm i.d.) filled with 0.05% polyvinylpyrrolidon K90 (Fluka) in Kaissling SLR, that was inserted into the body of the larva, between the ligature filaments. Gustatory responses were made with a non-blocking preamplifier (Maes, 1977). Electrophysiological signals were obtained by making 2–3 s contacts between the recording electrode tip and the dorsal or terminal organ.

2.6. Gas chromatography-coupled dorsal organ recordings (GC-DO)

Olfactory receptors in the dorsal organ were employed to locate active product(s) among the constituents of the cold-trapped banana odour extracts. These were separated on a high-resolution capillary chromatography column in a gas chromatograph (HRGC 5300 Mega Series II, Carlo Erba Insruments, Italy) with an on-column injector. The fused-silica column was a DB-Wax (J&W Scientific, USA; 30 m, 0.25 μm film thickness, 0.25 i.d.) with H_2 as carrier gas at 0.8 ml/min, and temperature programmed from 40°C after 3 min at 8°C/min to 200°C, 5°C/min to 240°C, and held for 10 min. The column effluent was split (50:50, glass Y-splitter) between the flame ionization detector (FID) and the dorsal organ preparation. The water-jacketed glass tube bearing the airflow over the preparation (above) swept half of the column effluent to the larval preparation 56 cm away from a heated transfer line (240°C) in the wall of the chromatograph. A discriminator incorporated in the amplifier allowed us to sort impulses from noise in the AC signal recorded from the dorsal organ, and the impulse frequency of recorded action potentials was converted into a DC voltage with a frequency to voltage converter (time constant 1 s). This sensillum response to stimulation by products eluting from the chromatographic column was printed with FID response on a chart recorder, and simultaneously recorded in the computer equipped with GC-coupled electrophysiology software (Syntech, The Netherlands). Quantification of extract components by peak area was made using an integrator (SP4270, Spectra-Physics, Germany).

2.7. Gas chromatography-coupled mass spectrometry (GC-MS)

These analyses were conducted with a mass selective detector (5971A, Hewlett Packard, Switzerland; ionization energy 70 eV, temperature 280°C) linked to a HP 5890 Series II GC equipped with an on-column injector and the DB-Wax column described above which was temperature programmed from 40°C after 3 min at 8°C/min to 180°C, 5°C/min to 240°C, and held for 5 min with He as the carrier gas at a flow rate of 0.7 ml/min. The components of 2 μl of either the cold- or Porapak®-trapped banana odour extracts injected were identified by comparing the mass spectra of unknowns with those of standards in the computer-based library of the GC-MS associated HP Chemstation, and by comparison of retention times of unknowns with those of standards injected under the same conditions as the extract.

2.8. Behavioural bioassay

The behavioural responses of third instar larvae to volatiles were measured in a 7-mm high, 120-mm wide and 200-mm long (inside measurements) glass (4-mm thick) wind tunnel with a 3-mm thick 1.2% agar floor. A ventilator blew air at 0.1 m/s through an inlet funnel with a muslin exit to the tunnel face to achieve a laminar flow of 0.1 m/s. A 100-ml gas-wash-flask with the chopped banana or a 500-ml one with the test volatile(s) (Table 1) diluted in paraffin oil (Merck, spectroscopy grade) impregnated on a 150-mm diameter filter paper disk (No. 311612, Schleicher & Schuell, Germany) served as the odour delivery device. The entry channel of the gas-wash-flask was connected to a humidified (60% RH) air source (100 ml/min), and the exit to a vertically held Pasteur pipette the bent tip of which conveyed the volatiles on to the upwind agar floor via a hole in the floor of the tunnel. Larvae were removed from the medium during the first 24 h of the third instar (as for electrophysiological experiments) and starved for 2 h prior to tests in 7-ml glass vials on humidified filter paper. They were released individually from the tip of a camel hair brush

Table 1
Responses of individual third instar *Drosophila melanogaster* larvae in a shallow wind tunnel to the odour of fresh banana, to individual olfactory stimulants for the larvae within banana odour and their mixtures, and to two known *D. melanogaster* larval attractants, i.e., propionic acid and isoamyl acetate[a]

Treatment	Source quantity of synthetics (μg)	No. of larvae tested	No. of larvae responding
Fresh banana	~150 g	51	47 (a)
2-Heptanone	100	15	8 (bc)
Hexanol	100	14	6 (c)
Isoamyl alcohol	100	10	1 (d)
Hexyl acetate	100	10	0 (d)
2-Heptanone:isoamyl alcohol:hexyl acetate:hexanol	10:100:100:30	14	7 (c)
2-Heptanone:hexanol	100:100	14	12 (b)
2-Heptanone:hexanol	50:50	15	12 (b)
Propionic acid	100	15	11 (b)
Isoamyl acetate	100	15	11 (b)

[a] Banana odour was tested as released from about 150 g of freshly cut banana in a 100-ml gas-wash-flask, whereas individual volatiles and mixtures were released from filter paper in a 500-ml flask. Numbers of responding larvae followed by different letters are significantly different (chi-square test and comparison of adjusted residuals).

at 80 mm from the odour source in the centre of the wind tunnel positioned in the middle of a 25-mm wide odour plume (as visualized using $TiCl_4$). Each larva first underwent a control run with a flask containing a filter paper disk treated with paraffin oil alone, and immediately afterwards with the test substance. Before proceeding with the following larva, the agar floor was rinsed with water to remove any contaminants. Responding larvae were those which crossed a line within the 12-mm wide plume at 20 mm from the odour source.

3. Results

3.1. Electrophysiology on the dorsal organ

Stimulation of the dorsal organ with the bouquet of cold-trapped banana volatiles evoked a multicellular response (Fig. 2), and tests with some 40 synthetic volatiles confirmed the organ's sensitivity to a range of products. Such recordings were made with 11 larvae, but due to the varying life-spans of the different preparations, all substances could not be tested on any one. Active substances were butanol, pentanol, hexanol, *trans*-2-hexen-1-ol, heptanol, ethyl acetate, hexyle acetate, *trans*-2-hexenyl acetate, 2-ethylhexyl acetate, hexyl butyrate, *trans*-2-hexenal, *cis*-3-hexenal, propionic acid, cyclohexanone and 2-heptanone. Hexanol evoked the most consistent response in all preparations, followed by *trans*-2-hexen-1-ol and heptanol, where a 1-μg source dose (about 340 pg/ml of air reaching the preparation) sufficed. Most of the other substances evoked responses at least once on the preparations tested, but responses were sometimes absent at any dose. The cell of the dorsal organ which responded to hexanol, characterized by an intermediate amplitude spike, also responded to *trans*-2-hexen-1-ol but with a lower increase in frequency (Fig. 3).

3.2. Gas chromatography-coupled dorsal organ recordings (GC-DO)

The dorsal organ detected a number of chemostimulants when it was used as an on-line detector in the analysis of the banana volatile extract by gas chromatography (n=4 runs, Fig. 4). Spike frequency increased with the elution of 2-heptanone (peak 1) and hexanol (peak 4). The amount of product eluting from the column was about 0.2 ng 2-heptanone and about 0.6 ng hexanol, equivalent to about 5 and 14 pg/ml of air, respectively, reaching the preparation. Two other banana odour constituents were active in the analysis presented, i.e., isoamyl alcohol (peak 2) and hexyl acetate (peak 3; Fig. 4). The response profiles varied between the larvae used in the four GC-DO analyses: 2-heptanone and hexyl butyrate (not shown in Fig. 4) were only active once, hexanol and isoamyl alcohol twice, and hexyl acetate thrice. All five products were identified based on matching mass spectra and Kováts' indices (Kováts, 1965) with synthetics in GC-MS analyses of the cold-trapped and Porapak® banana odour extracts (the profile of constituents was similar in both).

3.3. Terminal and dorsal organ responses to olfactory and gustatory stimuli

The electrophysiological responses of the dorsal and terminal organ receptors were also investigated to both olfactory and gustatory stimuli with one larval preparation. When the same recording system as that for the dorsal organ olfactory responses was applied to the terminal organ, no responses were obtained to stimulation

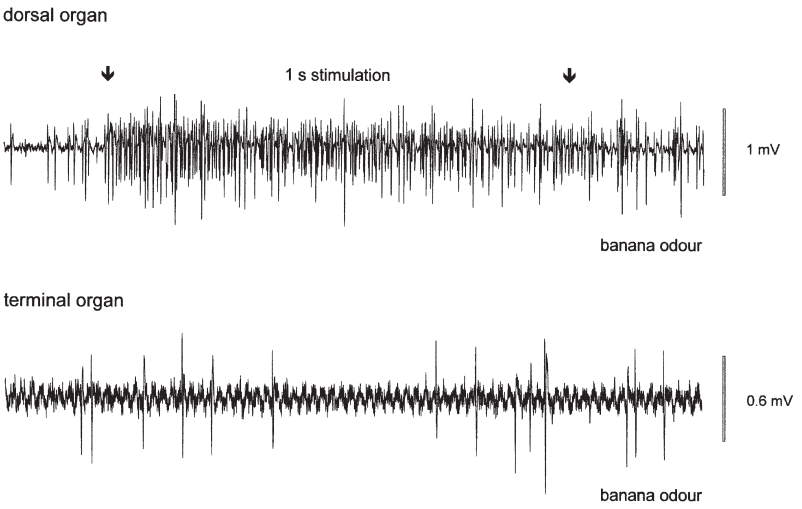

Fig. 2. Responses of receptors within the dorsal and terminal organs of third instar *Drosophila melanogaster* larvae to 1 s stimulation (between bold arrows) with a cold-trapped bouquet of banana volatiles. Different receptors within the dorsal organ respond with an increase in frequency well above the spontaneous firing rate recorded before stimulation, whereas spontaneously firing terminal organ receptors show no response.

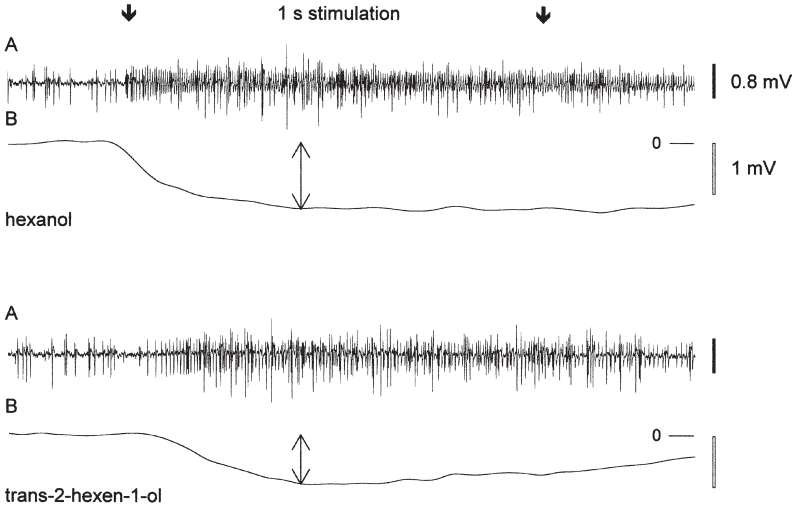

Fig. 3. Electrophysiological responses of receptors within the dorsal organ of a *Drosophila melanogaster* third instar larva to a 1 s stimulation with hexanol and *trans*-2-hexen-1-ol (10 μg source doses); (A) in each case is the action potential high-pass filtered response from the responding receptors, and (B) the low-pass filtered receptor potential response. The bold arrows delimit the start and end of stimulation; the zero level in (B) is the resting potential; and the light arrows in (B) represent the maximum receptor potential amplitude which is a 1.3 mV rise for hexanol and 1 mV for *trans*-2-hexen-1-ol. An olfactory receptor cell characterized by a spike of intermediate amplitude predominates in the response to both products, but fires at a higher rate at the start and end of stimulation to hexanol. Correspondingly, the half time of rise of the receptor potential is shorter and the half time of fall (return) is longer for hexanol.

with the banana odour bouquet (Fig. 2), hexanol, 2-heptanone, propionic acid and isoamyl acetate. By contrast, high frequency multicellular responses were recorded in response to touching the terminal organ with a wide-tip-opening electrode bearing either 100 mM KCl or 10% grapefruit juice in 100 mM NaCl (Fig. 5). NaCl was chosen as the electrolyte in which to deliver the fruit juice to the terminal organ sensilla as it elicited only a weak response on its own. Touching the dome of the dorsal organ with wide-tip-opening electrodes filled with either 100 mM KCl or NaCl elicited no responses comparable to those obtained by similar stimulation of the terminal organ.

3.4. Behaviour

Drosophila larvae showed odour conditioned upwind responses in the wind tunnel (Table 1). They responded best to the odour of freshly cut banana and, as with other attractants tested here, the larvae frequently located the Pasteur pipette tip which served as the odour source. Four banana odour constituents identified as chemosti-

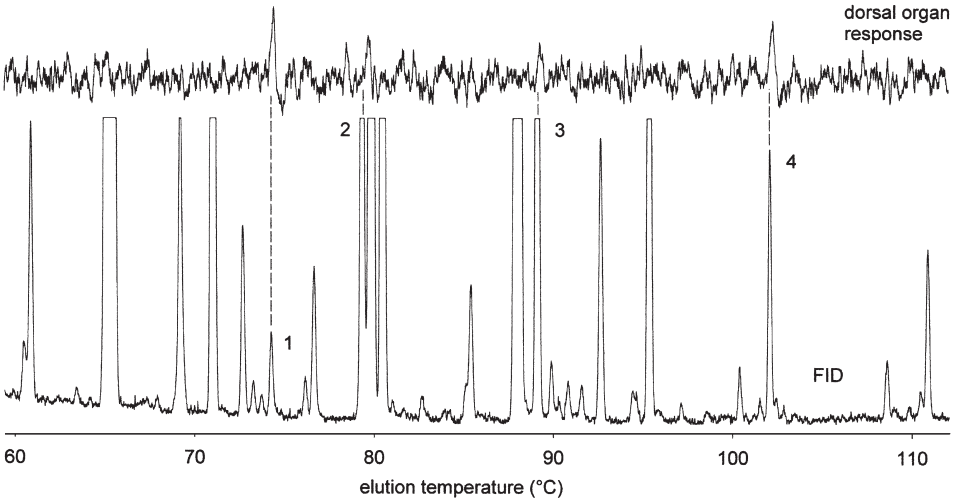

Fig. 4. Gas chromatography coupled to a larval *Drosophila melanogaster* dorsal organ electrophysiology preparation for the analysis of chemostimulants in a cold-trapped extract of banana odour. The banana volatiles were separated using a temperature programmed high resolution capillary column with one half of the effluent conveyed to the flame ionization detector of the chromatograph (FID, lower trace) and the other half swept over a nearby preparation of the larval dorsal organ (see text for details). The upper trace is the frequency modulated response of the dorsal organ to eluting chemostimuli converted to a DC voltage, i.e., a total spike frequency plot. Four active constituents of the extract were identified: 2-heptanone (peak 1) and hexanol (4) as the most active, and isoamyl alcohol (2) and hexyl acetate (3) as less active. Other constituents of the extract causing an increase in spike frequency were not identified due to the insufficient amounts present.

mulants in GC-coupled dorsal organ recordings (above), i.e., 2-heptanone, hexanol, isoamyl alcohol and hexyl acetate were also tested in the shallow wind tunnel. Of these, only 2-heptanone and hexanol proved attractive on their own, but less so than the known *Drosophila* larva attractants, propionic acid and isoamyl acetate (Ayyub et al., 1990) at the same source dose (Table 1). However, a 1:1 mixture of 2-heptanone and hexanol at either a 50- or 100-μg source dose of each proved as attractive as propionic acid or isoamyl acetate at a source dose of 100 μg on their own. When isoamyl alcohol and hexyl acetate were added to 2-heptanone and hexanol in the proportions found in the banana bouquet they did not increase the attraction of the 2-heptanone and hexanol blend.

4. Discussion

4.1. Dorsal organ electrophysiology

The electrophysiological responses obtained here following stimulation of the dorsal organ with banana volatiles confirms the olfactory function assigned to it based on ultrastructure studies on *Musca* (Chu and Axtell, 1971; Chu-Wang and Axtell, 1972a), *Drosophila* (Singh and Singh, 1984) and *Hylemya* (Honda and Ishikawa, 1987a) larvae. The multicellular response recorded from the dorsal organ to banana odour is clearly different from that evoked by single constituents of the odour, for whereas the former evoked responses of cells of very different amplitudes, the response to hexanol or *trans*-2-hexen-1-ol for example is characterized by modulation of the frequency of just one unit. Despite the extensive branching of the sensory neurones beneath the dome described by earlier investigators, our recordings show that the site of electrode placement within the dome is a critical factor in determining the profile of the response from each preparation; the tip of the electrode most probably reached the dendritic branching area. Responses to some electrophysiologically active substances were absent from certain preparations used for screening among synthetics for chemostimulants and in GC-DO analysis of banana odour. No response to isoamyl acetate was recorded in any of the preparations, either as a synthetic presented on its own, or as a constituent of the extracts in GC-DO analysis of the banana bouquet. The selectivity of the receptors for certain natural products is demonstrated by the fact that responses were recorded for only a few fractions in any given GC run, despite the fact that the extracts contained at least 100 products. Whole banana odour extract presented as an odour puff invariably evoked a response, suggesting that the bouquet of the fruit contained a sufficient amount of different products to activate a certain number of receptors in each preparation independent of the site of electrode entry. Furthermore, biological activity of hexanol in a high number of the preparations would suggest that receptors for this type of product are particularly well represented. Studies on the genetics of olfactory responses of *Drosophila* larvae using behavioural assays (Cobb et al., 1992) suggest the existence of at least two types of receptors for alcohols.

The electrophysiological responses reported here for

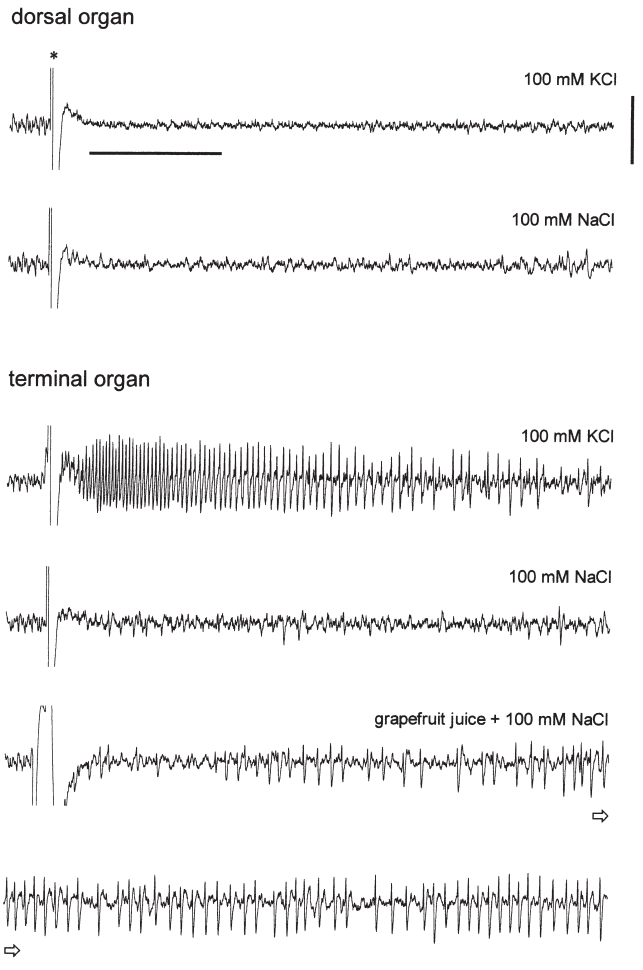

Fig. 5. Dorsal and terminal organ responses of a *Drosophila melanogaster* third instar larva upon contact with a 12–15 μm tip diameter glass electrode containing either 100 mM KCl or NaCl, and of the terminal organ to 10% grapefruit juice in 100 mM NaCl. Note the near absence of a response by the dorsal organ to contact with either of the salts, compared with the strong response of the terminal organ to KCl. NaCl did not elicit a comparable response from the exposed sensilla on the terminal organ, but served as an electrolyte for recording the multicellular response to grapefruit juice (bottom trace divided in two). The asterisk in the first trace marks the artefact common to these high-pass filtered recordings arising from making contact with the sensory organs; the non-blocking preamplifier brought the recording back to base level within about 15 ms of contact. The vertical bar represents 1 mV and the horizontal one 200 ms.

the dorsal organ to butanol, ethyl acetate and cyclohexanone have already been recorded from individual basiconic sensilla on the adult *Drosophila* antenna (Siddiqi, 1991) and using the electroantennogram technique (Venard and Stocker, 1991), suggesting that receptors developed in the larval stage are again expressed in the adult stage. Evidence for the common expression of olfactory receptors between the life-stages of holometabolous insects exists: *trans*-methyl-isoeugenol which attracts carrot fly larvae *Psila rosae* (Jones and Coaker, 1979) acts as a strong stimulant for the antenna in the adult as well as being a field attractant (Guerin et al., 1983) and an oviposition stimulant for the fly (Städler and Buser, 1984); di-*n*-propyldisulfide which activates a receptor in the dorsal organ of larvae (Honda and Ishikawa, 1987b) and antenna of adult onion flies, *Hylemya antiqua* (Honda et al., 1987), also attracts the larvae (Mochizuki et al., 1989) and adults in the field (Dindonis and Miller, 1981), as well as serving as an oviposition stimulant (Ishikawa et al., 1978).

4.2. Behaviour

The behavioural data are coherent with the electrophysiology findings. The multicomponent odour of freshly cut banana proved the best attractant. A mixture of 2-heptanone and hexanol at 50 μg each also proved more attractive than either of these two products alone at twice the dose. These effects underline the contribution of mixtures of products capable of evoking responses in a range of receptors within the dorsal organ to permit best localization of the odour source by the larvae. Freshly cut banana odour is even more complex than that exemplified by gas chromatographic analysis of the volatile collection. It contributed to an increase of some 100 ppm CO_2 in the odour plume from the fruit (as measured by a Binos IR CO_2 detector, Leybold-Heraeus, Germany). The CO_2 may have contributed to the attraction of the *Drosophila* larvae to the banana, just as larvae of other insect species are attracted to this ubiquitous respiratory product (Rasch and Rembold, 1994).

Many investigators have analysed the behavioural responses of *Drosophila* larvae to volatiles for the purposes of characterizing olfactory mutants. These tests are based on responses shown by larvae to point sources in a Petri dish from which the test chemical diffuses throughout the chamber (Aceves-Piña and Quinn, 1979); mg doses of products are frequently used, giving rise to relatively high doses of products in the air. In this study the volatiles tested were presented in a laminar air flow from a gas-wash-flask, permitting the establishment of a discrete plume with pg amounts of products per unit volume of air down the centre of the wind tunnel. The larvae moved upwind to attractive chemicals. When the border of the plume was encountered during such upwind crawls, the larvae made either sharp returns or were induced to swing the head and first body segments in the air only to bring the whole body down again on the agar within the plume. Occasionally, when the border of the plume was completely overrun, they crawled back to the plume. Similar responses have been described for carrot fly larvae (Jones and Coaker, 1979). Testing larvae individually permits a clear discrimination between responders and nonresponders, enabling the characterization of individuals and siblings.

4.3. Sensory roles of dorsal and terminal organs

The olfactory function of the dorsal organ has been confirmed by the fact that compounds which evoke responses from receptors within it proved attractive in the wind tunnel assay. It should be noted that the banana volatiles isoamyl alcohol and hexyl acetate which proved unattractive but which were shown to activate dorsal organ receptors in GC-DO analysis of banana odour, were present at much higher levels than 2-heptanone and hexanol in the odour extract. Clear responses obtained with wide-tip-opening electrodes containing phagostimulants such as KCl and grapefruit juice implicate the terminal organ in gustation. Such responses were absent in similar stimulation of the dorsal organ. By comparison with the response of the terminal organ to KCl, the lack of response to NaCl could be due either to a lack of receptor(s) for the salt or to inhibitory effects. An argument could be made to support the latter premise since Lilly and Carlson (1990) have shown that *Drosophila* larvae avoid agar substrates with high NaCl concentrations. This could have a survival value for a terrestrial arthropod such as *Drosophila* larvae which are particularly susceptible to desiccation. By contrast, the strong response to KCl by the terminal organ sensilla fits with the fact that *Drosophila* larvae feed on microorganisms on decomposing vegetable matter where KCl is the predominating salt, present at concentrations up to 200 mM.

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