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C. Giovanni Galizia · Silke Sachse · Hanna Mustaparta

Calcium responses to pheromones and plant odours in the antennal lobe of the male and female moth *Heliothis virescens*

Abstract In male moths, the primary olfactory integration centre, the antennal lobe, consists of two systems. The macroglomerular complex processes pheromone information, while the ordinary glomeruli process plant odour information. Females lack a macroglomerular complex. We measured the spatial representation of odours using in-vivo optical recording. We found that: (1) pheromone substances elicited activity exclusively in the MGC. No response was found in female antennal lobes. (2) Plant odours elicited combinatorial activity patterns in the ordinary glomeruli in both males and females. No response was found in the MGC of male moths. (3) A clean air puff often led to activity, in both males and females, suggesting that mechano-sensory information is also processed in the antennal lobe. (4) With an interstimulus interval of 5 or 10 s, strongly activated glomeruli were able to follow the temporal structure of the stimulus, while others lost their phase-locking. Some glomeruli showed ''off'' responses. These properties were odour dependent. This confirms and extends previous studies, showing the functional significance of the two subsystems for processing olfactory information. Pheromones are coded in a combinatorial manner within the macroglomerular complex, with each glomerulus corresponding to one information channel. Plant odours are coded in an across-glomeruli code in the ordinary glomeruli.

Key words Optical imaging · Calcium green · Spatial coding · Olfactory representation · Repetitive stimulation

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Abbreviations *ISI* interstimulus interval · *MGC* macroglomerular complex · *Z11–16:AC* Z-11-hexadecenylacetate · *Z11–16:AL* · Z-11-hexadecenal · *Z11–16:OH* *Z*-11-hexadecenol · Z9–14:AL *Z*-9-tetradecenal

Introduction

The olfactory system in many vertebrate and insect species can be subdivided into a ''main'' and an ''accessory'' part, involved in detection of food odours and pheromones, respectively. In male moths, the primary olfactory integration centre, the antennal lobe of the deutocerebrum, relays both kinds of information into two separate systems of glomerular structures (reviewed by Homberg et al. 1989; Masson and Mustaparta 1990; Hildebrand and Shepherd 1997). The macroglomerular complex (MGC) is involved in processing pheromone information, as demonstrated by electrophysiological recordings combined with stainings of antennal lobe neurones (Boeckh and Boeckh 1979; Christensen and Hildebrand 1987; Christensen et al. 1989, 1991, 1995a; Hansson et al. 1991). The antennal lobe projection neurones responding to olfactory stimulation with pheromones have dendritic arborizations exclusively or mainly in the MGC. The input to the MGC by receptor neurones responding to pheromones is demonstrated by staining of sexually dimorphic sensilla (Koontz and Schneider 1987; Christensen et al. 1995b) and also by stainings combined with recordings from the cut tip of olfactory sensilla, showing projections mainly in the MGC (Berg et al. 1998; Hansson et al. 1992, 1995). A functional subdivision of the MGC has been demonstrated by electrophysiological recordings from receptor and antennal lobe neurones combined with stainings in several species of moths and other insects (Christensen et al. 1991; Hansson et al. 1991, 1995; Berg et al. 1998; Vickers et al. 1998).

Well separated from the MGC are the numerous ordinary glomeruli that seem to be involved in the processing of general odours. The antennal lobes of

female moths lack an MGC. It consists entirely of "ordinary" glomeruli, and a "modified glomerular complex", which is possibly involved in host-odour recognition (King et al. 2000). In the (female) honeybee, optical recordings from the antennal lobe during stimulation with plant odours have demonstrated a spatial pattern of activity characteristic for each odour. These patterns are conserved within the species, and can be mapped to identified glomeruli (Galizia et al. 1999a; Sachse et al. 1999).

In the tobacco budworm moth, *Heliothis virescens*, receptor neurones responding specifically to insect- or plant-produced compounds have been identified. In males, four sex-specific receptor neurone types have been classified, each projecting to one of the four MGC compartments (Berg et al. 1995, 1998). Neurones tuned to the major pheromone component, *Z*-11-hexadecenal (Z11–16:AL), project to the largest compartment, cumulus, whereas the neurones responding selectively to the second pheromone component, *Z*9-tetradecenal (Z9–14:AL) project to the dorso-medial compartment. The other two neurone types are each tuned to one of the interspecific signals, *Z*11-hexadecenol (Z11–16:OH), and *Z*11-hexadecenylacetate (Z11–16:AC), produced by sympatric females, and project to one of the smaller ventral compartments, respectively. In this species there seems to be a correspondence between input and output of the MGC, i.e. that antennal lobe projection neurones responding to one of the four compounds have at least one dendritic arborisation in the compartment receiving that particular information (Berg et al. 1998; Vickers et al. 1998). In the female *H. virescens* several types of plant odour receptor neurones have been identified by the use of gas chromatography linked to recordings from single neurones (Røstelien et al. 2000a, b). For each neurone type, selective responses were demonstrated to one or two compounds, monoterpenes or sesquiterpenes, of the volatiles naturally produced by plants. One frequently occurring receptor neurone type responded selectively to the sesquiterpene germacrene D. Another neurone type responded selectively to the monoterpenes trans-β-ocimen and β-myrcene and a third type selectively to linalool, all present in host and non-host plants. These results raise the question of how these odours are represented in the antennal lobe. Neuron staining, combined with the electrophysiological recordings, has not yet been performed. In the present study optical recordings from the antennal lobes of females and males have been used to study the spatial pattern of activity elicited by antennal stimulation with odours. We tested the four insect produced compounds, three of the four identified plant compounds as well as naturally produced plant volatile mixtures and an isolated fraction of a plant extract.

The results confirm and extend results from electrophysiological recordings of pheromone receptors and antennal lobe projection neurones, showing (1) the significance of the two subsystems, the MGC and the ordinary glomeruli, for processing information about insect and plant produced odours, (2) a functional subdivision of the MGC compartments in processing information about intra- and interspecific insect produced signals, and (3) specific patterns of activity in the ordinary glomeruli by stimulation with different odours.

Materials and methods

Animal stocks

Heliothis virescens (Lepidoptera: Noctuidae) pupae, originating from a lab culture, were kindly provided by Novartis Crop Protection, Basel, Switzerland. Male and female pupae were separated into different containers and kept in an incubator on a phase-shifted light-dark 14 h:10 h photoperiod at 23 °C. Every day, moths that had emerged were placed in a separate container marked with the date. Animals were allowed to feed on honey-water. Most animals were studied 1–3 days after emergence.

Animal preparation and staining

The moths were prepared in a way similar to that for standard electrophysiological recordings. The animal was fixed into a Plexiglas stage and the antennae were fastened in the desired position with utility wax (Kerr). Scales were removed; a hole was cut into the head cuticle in order to expose the brain. Mouthparts, glands and tracheae were gently removed. The brain was kept wet at all times with Ringer solution containing (mmol l^{-1}): 130 NaCl, 6 KCl, 4 $MgCl_2$, 5 $CaCl_2$, 160 sucrose, 25 glucose, 10 HEPES, pH 6.7, 500 mosmol l^{-1}; all chemicals from Sigma. The brain was then floated in dye solution (calcium-green-2 AM, Molecular Probes; 50 µg dye was first dissolved in 50 µl Pluronic in DMSO and then diluted in 950 µl Ringers saline). After 1 h, the brain was rinsed in fresh Ringer solution, covered with a cover slip, and placed under the microscope. During the experiment, a constant Ringer flow was applied (1 ml min^{-1}, 22°C).

Odours used and stimulus application

The animals' antennae were constantly puffed with a clean air stream, which was replaced by an odour-laden air stream for stimulation. Controlled amounts of odours were placed on filter paper in glass cartridges. Odours were dissolved in 1-hexane. Stimulus concentration was varied by using different dilutions, resulting in different effective amounts on the filter paper (0.2 µg, 2 µg, 20 µg in the case of the pheromones, and 0.01 µl, 0.1 µl and 1 µl for plant odours). These effective amounts are referred to as concentrations in this paper. Different amounts lead to different stimulus intensities. A clean glass cartridge was used as control.

The insect-produced substances used for olfactory stimulation were: two of the six identified pheromone components, *cis*-11-hexadecenal (Z11–16:AL) and *cis*-9-tetradecenal (Z9–14:AL), and two intraspecific disruptants, *cis*-11-hexadecenyl acetate (Z11–16:AC) and *cis*-11-hexadecenol (Z11–16:OH). These compounds, provided by Dr. JG Tumlinson, USDA, Gainesville, Florida, had a purity above 99% (checked by GLC). Plant odours tested were: (1) *Pipera cubeba* oil (provided by Dr. G Schmaus, Dragoco, Holzminden, Germany), which is a sesquiterpene fraction containing mainly sesquiterpenes including germacrene D, but also small amounts of many other compounds (including linalool, *cis*-/*trans*-β-ocimen, and β-myrcene); (2) headspace of sunflower and tobacco plants (flower and leaves); (3) synthetic materials of β-ocimen, *trans*-/*cis*-β-myrcene (ratio 66:33); and (4) the pure substances linalool, (racemic mixture with purity 95–98%, supplied by Firmenich, Geneva), citral, hexanal and 1-hexanol (Sigma, purity 95%). Not all odours were tested in all animals. Generally, each odour was delivered two times in a row, with an

inter-stimulus interval (ISI) of 1 min. The entire stimulus sequence comprising all odours was repeated between two and four times in each animal.

Stimulus timing was controlled in different ways in different experiments. In most experiments, a computer gave a characteristic series of sounds, which allowed the experimenter to press a button. The button activated the stimulus controller which controlled two solenoid valves: one was closed, thus stopping a continuous air stream, and one was opened, thus delivering the stimulus to the animals' antennae. Stimulus duration was electronically controlled to 1 s. In later experiments, the computer also directly controlled stimulus timing. Stimulus application also differed between experiments. In most experiments, the clean air and the odour came from two separate cartridges. A stimulus thus consisted both in a mechanosensory component (change of air-flow direction) and an olfactory components. In later experiments the odour-laden air was puffed into the continuous air stream (see Results). Both sets were used in the analysis.

Calcium measurements

Imaging was done using a Till-Photonics imaging system (http://www.till-photonics.com). Monochromatic excitation light was 488 nm, dicroic: 510 nm, emission: BP515–565. Measurements were made with an upright Axioscop microscope (http://www.zeiss.com), using a Leica 20 × LD NA = 0.6 air objective (http://www.leica.com). Pixel image size was 2.4 μm × 2.4 μm, obtained by 2 × 2 binning on chip. For each stimulus, a series of either 30 or 40 frames was taken, with exposure times of between 166 ms and 250 ms for the single frames, and frequencies of either 3 Hz or 4 Hz. Light was shut off between frames. ISI was 1 min, i.e. the animal was not exposed to light for about 50 s between measurements.

Upon stimulus delivery, patterns of intracellular calcium increase appeared in the antennal lobe of *H. virescens*. We measured 35 males and 17 females. Of these, we got consistent responses to stimulation with plant odours in 13 males and 10 females. Lacking a three-dimensional atlas of the "ordinary" heliothine glomeruli, we were unable to relate the glomerular responses of plant odours to identified glomeruli, neither in males nor in females. Therefore, it was impossible to compare these results from different animals at the level of individually identified glomeruli.

We saw responses to pheromone components in three males. Results were considered consistent when repeated stimulation with the same odour led to the same activity pattern, and different odours led to different activity patterns. In the males responding to pheromones we were able to relate the activity to the known subcompartments of the MGC. These results were confirmed in all three animals measured.

Data processing: false colour display and time-courses

Raw data were median-filtered for noise reduction (filter size 3 pixels in two spatial and one temporal dimension). Signals were calculated as $\Delta F/F$, where the mean of all frames measured was used as F. Measurements were then corrected for global bleaching and lamp noise by subtracting the median frame value for each frame. Then the mean value of frames 3–5 (i.e. before stimulus) was subtracted from the time-course of each pixel. This subtraction corrects for shifts that arise from differential bleaching in different areas, and thus allows for better comparison of stimulus response time-courses. This correction is routinely used in our lab. However, when there is spontaneous activity, the following must be kept in mind in judging the time-courses: since all curves now go through zero shortly before stimulus, in control trials with strong background activity, this gives an impression of either excitation or inhibition being stimulus-correlated, which would be an erroneous interpretation (e.g. see air trials in Fig. 4, or curves for non-responsive glomeruli A and B in Fig. 2). With this caveat in mind, the chosen analysis yields the best description of stimulus-evoked calcium activity in the antennal lobe.

For false-colour display, at each pixel a square of 12 pixels × 12 pixels was averaged in its time-course; the difference between the maximum in the time interval of 3 s after stimulus onset and the minimum in the 2 s interval before stimulus onset was calculated for this curve, and the value attributed to the pixel. For time-courses, squares of 15 pixels × 15 pixels (corresponding to 30 μm side length, which was large enough to include the glomerulus, and small enough to exclude neighbouring maxima) were placed onto the activity maxima of the false-colour coded maps. Their time-courses were averaged, and the resulting values plotted against time.

Data processing: cluster analysis

For the cluster analysis (Fig. 7), 18 different glomeruli were chosen in the antennal lobe of an individual. The response to each odour was described as a vector of 18 dimensions, where the value in each dimension corresponded to the response maximum in that glomerulus after stimulus application (in a time interval of 3 s). Vectors were normalised to a unitary length of 1. These responses were clustered with Ward's method using the JMP software (http://www.sas.com). The analysis in other individuals (with a different number of glomeruli chosen) gave similar results. Clustering across individuals is not possible without morphologically identifying the glomeruli. This was not possible because no morphological atlas of the ordinary glomeruli for *H. virescens* is available to date. Furthermore, in the calcium-imaging experiments, individual glomeruli are not visible unless additionally labelled with another dye (Galizia et al. 1999a; Sachse et al. 1999).

Periodic odour stimulation

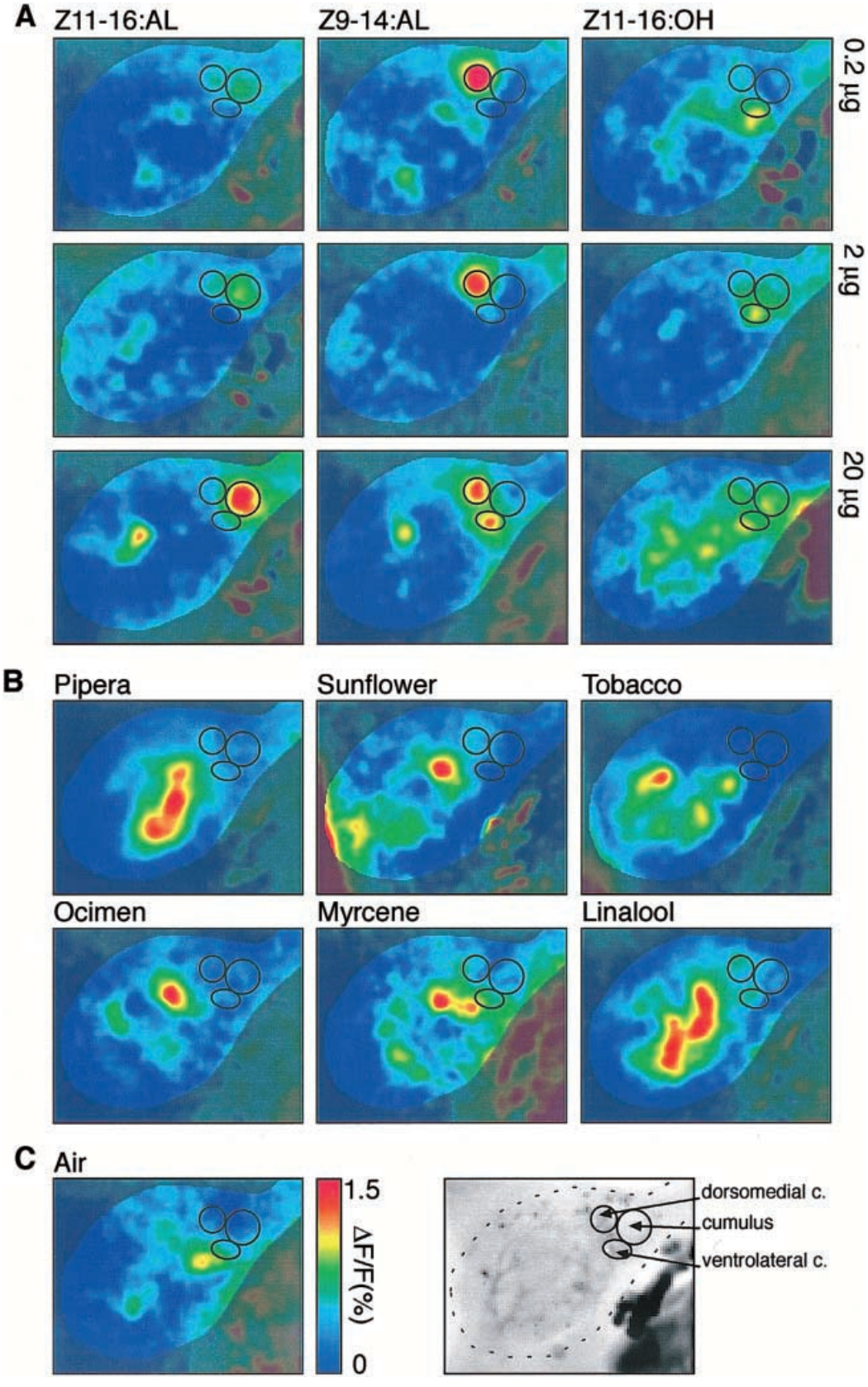
In these experiments, odour puffs were delivered either every 5 s (0.2 Hz) or every 10 s (0.1 Hz), for a total duration of 100 s. Acquisition rate was 3 frames/s. $\Delta F/F$ was calculated as for single-stimulus trials. False-colour coded images were calculated by averaging 3 frames after each stimulus, and subtracting the average of 3 frames before each stimulus. The resulting frame was scaled to its own minimum and maximum. In this way, in the false-colour-coded images red regions are those that consistently responded to the repeated stimulation, blue regions those that consistently reduced their activity to the repeated stimulation. Time-courses were calculated from square areas, and band-pass filtered in order to remove the bleaching effect and high-frequency noise.

Results

Male moths: responses to pheromone components

Specific responses to pheromones were limited to the MGC region of the antennal lobe. Responses to Z11–16:AL, Z9–14:AL and Z11–16:OH at three concentration levels are shown in Fig. 1. Each substance consistently activated a particular, unique spot, which can be identified by taking into account the orientation of the antennal lobe. These results were confirmed in all animals with responses to pheromone components.

In order to compare the responses, we plotted the time-courses of the calcium signals for the three visible glomeruli in the MGC together with the responses in two glomeruli in the rest of the antennal lobe (Fig. 2). The first three rows show responses in the MGC: Z11–16:AL elicited a strong response in the cumulus, Z9–14:AL in the dorsomedial, and Z11–16:OH in the ventral glomerulus, while there was no convincing



response to Z11–16:AC, apart from a very weak and concentration independent response in the dorsomedial and ventrolateral glomeruli (stimulus concentration is given by the line style). Furthermore, stimulation with high concentrations of Z9–14:AL also led to intracellular calcium increase in the ventrolateral compartment.

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Fig. 1A–C False-colour coded spatial response patterns to odour stimulation in the antennal lobe of a male *Heliothis virescens* moth. The area outside the antennal lobe has been shaded. **A** Responses to components of the sexual pheromone system, *Z*-11-hexadecenal (Z11–16:AL), Z-9-tetradecenal (Z9–14:AL), Z-11-hexadecenol (Z11–16:OH), at the three concentrations 0.2 μg, 2 μg and 20 μg. The positions of the cumulus, the dorsomedial and the ventrolateral compartment are shown with *circles* to simplify comparing the patterns. Note that Z11–16:AL elicits a selective response in the cumulus, Z9–14:AL elicits a selective response in the dorsomedial compartment, and Z11–16:OH elicits a response in the ventrolateral compartment. **B** Responses to different plant odours, all at a concentration of 1 μl. Each odour elicits a characteristic and unique pattern of different activated glomeruli. Note the similarity of the ocimen and myrcene responses, and the overlap between pipera (which contains linalool) and linalool itself. There are no responses to any of the odours in the macroglomerular complex (MGC). **C** *Left*: response to a control stimulation with an empty cartridge (air), and false-colour code valid for the entire figure; *right*: morphological view of the antennal lobe, where the border of the antennal lobe can be seen

In the non-MGC part of the antennal lobe, some glomeruli occasionally showed responses to stimulation (fourth row in Fig. 2). These responses were weak and independent both of odour quality and odour concentration (0.5% $\Delta F/F$). They were sometimes present and sometimes absent from the response pattern. These responses are most likely due to mechanosensory input to the antennal lobe, since they also appeared with air-control stimulation. We also observed a high degree of spontaneous activity in some glomeruli of the antennal lobe (e.g. bottom row in Fig. 2, also in the range of 0.5% $\Delta F/F$). This activity is not related to the stimulus, leading to the curves going both up or down. The correspondence of all curves just before stimulus is a consequence of the way in which the data are normalised, and has no biological significance (see Materials and methods).

We tested the pheromones at three concentrations (0.2 μg, 2 μg, 20 μg). Furthermore, each stimulus was given twice in a row, with an interstimulus interval of 1 min. We found that different glomeruli responded in different ways to these parameters. Three examples are given in Fig. 3 (compare with the curves in Fig. 2). The cumulus showed increasing response with increasing concentrations of Z11–16:AL. For the low and intermediate concentration, the response to the second stimulus was in the same range as for the first, but at 20 μg the response to the second stimulus was weaker than to the first. The response to Z9–14:AL in the dorsomedial compartment decreased with increasing concentration. Repeated stimulation led to a weaker response at all concentrations. A similar, though much less pronounced pattern was observed in the ventral region responding to Z11–16:OH.

Male moths: responses to plant odours

Stimulation with plant odours led to strong responses in the antennal lobe of male moths. Each odour elicited a unique pattern of activated glomeruli, and individual glomeruli participated in the patterns for different odours. Examples of spatial activity patterns are given in Fig. 1B. The time-courses of responses in four selected glomeruli of the non-MGC region of the antennal lobe are shown in Fig. 4B, while Fig. 4C shows the responses to the same stimuli in the three visible glomeruli of the MGC (dorsomedial compartment, cumulus and ventrolateral compartment). Both pipera and linalool generally led to several glomeruli being activated. The maximum response in glomeruli D and B was over 2% $\Delta F/F$ for pipera, and about 1.5% $\Delta F/F$ for linalool. Tobacco elicited the strongest response in glomerulus A (1.6% $\Delta F/F$). Ocimen and myrcene led to responses around 1%, with the same glomerulus (glomerulus B) being the strongest.

Stimulation with plant odours did not activate the glomeruli in the MGC (Figs. 1B and 4C), with the exception of strong stimulation with linalool and pipera (which contains linalool), when signals below 0.5% $\Delta F/F$ could be observed (Fig. 4C).

Female moths: responses to plant odours and pheromones

As in the male, stimulation with plant odours also led to strong responses in the antennal lobe of female moths, with a combinatorial pattern of activated glomeruli. Examples of spatial activity patterns are given in Fig. 5. The time-courses of responses in four selected glomeruli are shown in Fig. 6.

Increasing odour concentration led to stronger responses in most cases, though there were exceptions for some glomeruli when stimulated with particular odours (see below). The response to ocimen at the three concentrations 0.01 μl, 0.1 μl and 1 μl led to an increase of the response in glomerulus E from below 1% $\Delta F/F$ to above 2% $\Delta F/F$ (Fig. 6, compare with open arrowhead in Fig. 5A). Glomerulus G also increased its activity, while glomerulus F was fairly stable around 1% (glomerulus F in Fig. 6 corresponds to the close arrowhead in Fig. 5A). Note, however, that glomerulus F also occasionally responds to the mechanosensory component of the stimulus alone (Fig. 6C). While glomerulus G responded in a concentration-dependent way to myrcene, glomerulus E did not. To pipera, the strongest responses could be observed at intermediate concentrations. This behaviour was observed in all animals. However, it was not possible to identify the glomeruli, and thus to show that homologous glomeruli had the same concentration-dependent responses.

The temporal response pattern was not equal for odours and glomeruli. For example, glomerulus H was the strongest glomerulus when responding to pipera, always increasing in activity right after stimulus onset, and reaching its maximum response about 1 s after stimulus offset (or 2 s after stimulus onset). The same glomerulus, when responding to linalool was always

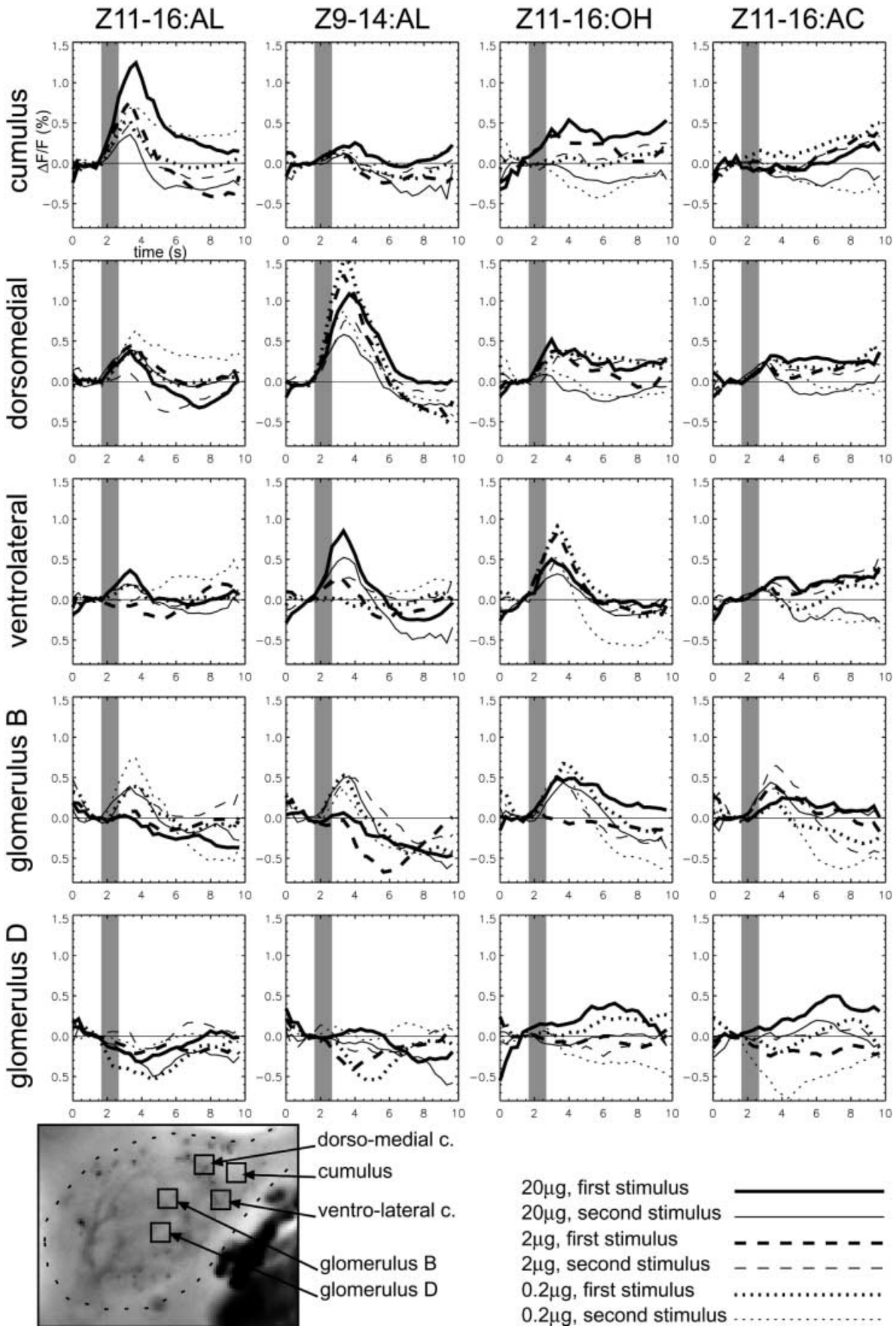
Z11-16:AL
Z9-14:AL
Z11-16:OH
Z11-16:AC
cumulus
dorsomedial
ventrolateral
glomerulus B
glomerulus D
ΔF/F (%)
time (s)
dorso-medial c.
cumulus
ventro-lateral c.
glomerulus B
glomerulus D
20µg, first stimulus
20µg, second stimulus
2µg, first stimulus
2µg, second stimulus
0.2µg, first stimulus
0.2µg, second stimulus

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Fig. 2 Glomerular calcium responses to pheromone stimulation in the antennal lobe of a male *H. virescens*. Signals are shown as $\Delta F/F$ (in %) over time (s). Stimulus (1 s) is given by the *grey shading*. The rows show the responses in five glomeruli (from top to bottom: cumulus, dorsomedial and ventrolateral glomerulus in the MGC, and two glomeruli from the rest of the antennal lobe) to four different odours [columns, from left to right: Z11–16:AL, Z9–14:AL, Z11–16:OH and Z-11-hexadecenylacetate (Z11–16:AC)]. Each plot contains six curves, representing six separate measurements, with three different concentrations: 0.2 μg per cartridge (*dotted lines*), 2 μg per cartridge (*dashed lines*) and 20 μg per cartridge (*continuous lines*). For each concentration, two measurements are shown, which were taken sequentially at an interval of 1 min. The *first stimulus* is shown as a *thick line*, *the second as a thin line*. The positions of the glomeruli chosen are given at the bottom left. It is the same animal as in Fig. 1, so that the time-courses can be compared to the spatial activity pattern. Z11–16:AL leads to a response in the cumulus, Z9–14:AL to a response in the dorsomedial and Z11–16:OH to a response in the ventrolateral compartment of the MGC. No response to Z11–16:AC is observed. See text for a more detailed description

late, though showing reliable responses, and this delay was even larger in the response to ocimen and myrcene (Fig. 6).

Stimulating with air alone, i.e. just applying the mechanosensory component of the stimulus, led to weak (only rarely above 0.5% in glomeruli in which an odour response could reach 2%) and inconsistent (sometimes present, and sometimes not), but very clear responses. However, some glomeruli of the antennal lobe never gave any response to air stimulation, and generally one glomerulus was the most prominent, and most frequent component of the air response (see Figs. 5 and 6 for one example). In most experiments, odour stimulation was achieved by switching between two cartridges (one clean for the continuous air stream, and one odour-laden, or empty for the air control), which were positioned in front of the animal. Therefore, a stimulus also consisted of the fact that the air was coming from a slightly different direction, which may account for a mechanical stimulation. In some experiments, however, the odour was puffed into a continuous air stream, so that both stimulus and continuous airflow were directed at the antennae through the same nozzle. In these animals, the response to air alone was still present (probably because puffing air into the continuous air stream changes the air flow pressure), but limited to a single glomerulus, and was much more reliable than with the previous stimulating arrangement (data not shown).

When stimulating with pheromone components we never saw responses in the female antennal lobe that were stronger than those observed to the mechanical component of the stimulus, i.e. to air-control stimulation.

Cluster analysis of glomerular responses

In order to test how consistent the responses were for repeated stimulation, we performed a cluster analysis on the glomerular responses. The results are shown in Fig. 7. The odours pipera, ocimen, and myrcene appear in clusters (there is one myrcene measurement in the ocimen cluster), with ocimen and myrcene being closer to each other than both are to pipera. Responses to air control form another cluster. One response to pipera, which was the first stimulus to this animal and a very weak response, also landed in the air cluster. Within the pipera cluster, two different concentrations formed subclusters.

The animal shown in Fig. 7 showed only very few responses to mechanosensory stimulation. A cluster analysis did not give satisfactory results in those animals where the mechanosensory component of the response was strong; the clusters did not form according to the odour stimulus given. This is because the response pattern to a particular odour sometimes included, and sometimes did not include the mechanosensory compo-

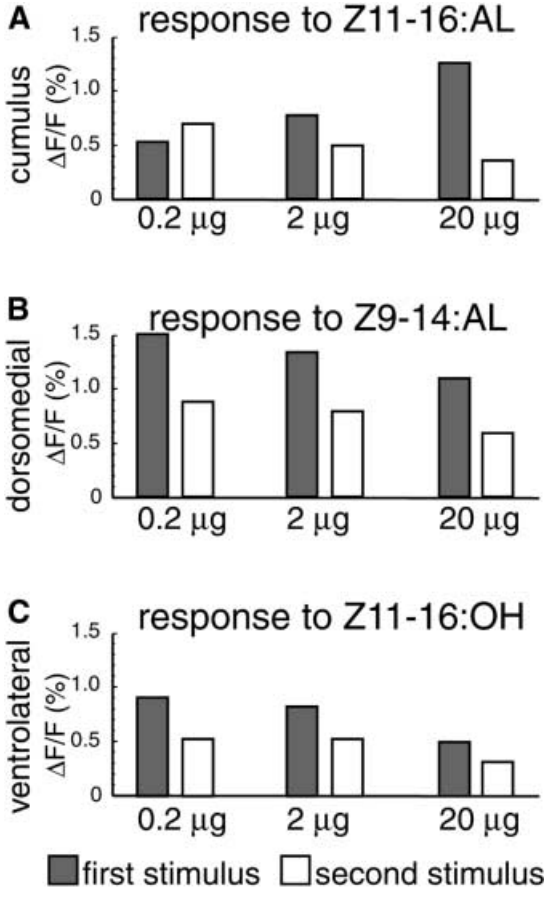

Fig. 3A–C Comparison of maximum responses for different concentrations (0.2 μg, 2 μg, 20 μg) and repeated stimulation in individual glomeruli of a male MGC. **A** Responses to Z11–16:AL in the cumulus. **B** Responses to Z9–14:AL in the dorsomedial compartment. **C** Response to Z11–16:OH in the ventrolateral glomerulus. In each pair of bars, the *filled bar* represents the maximum response to the first stimulus, the *open bar* the maximum response to the second stimulus (1 min later). Before stimulating with the higher concentration, the animal was exposed to several other odours

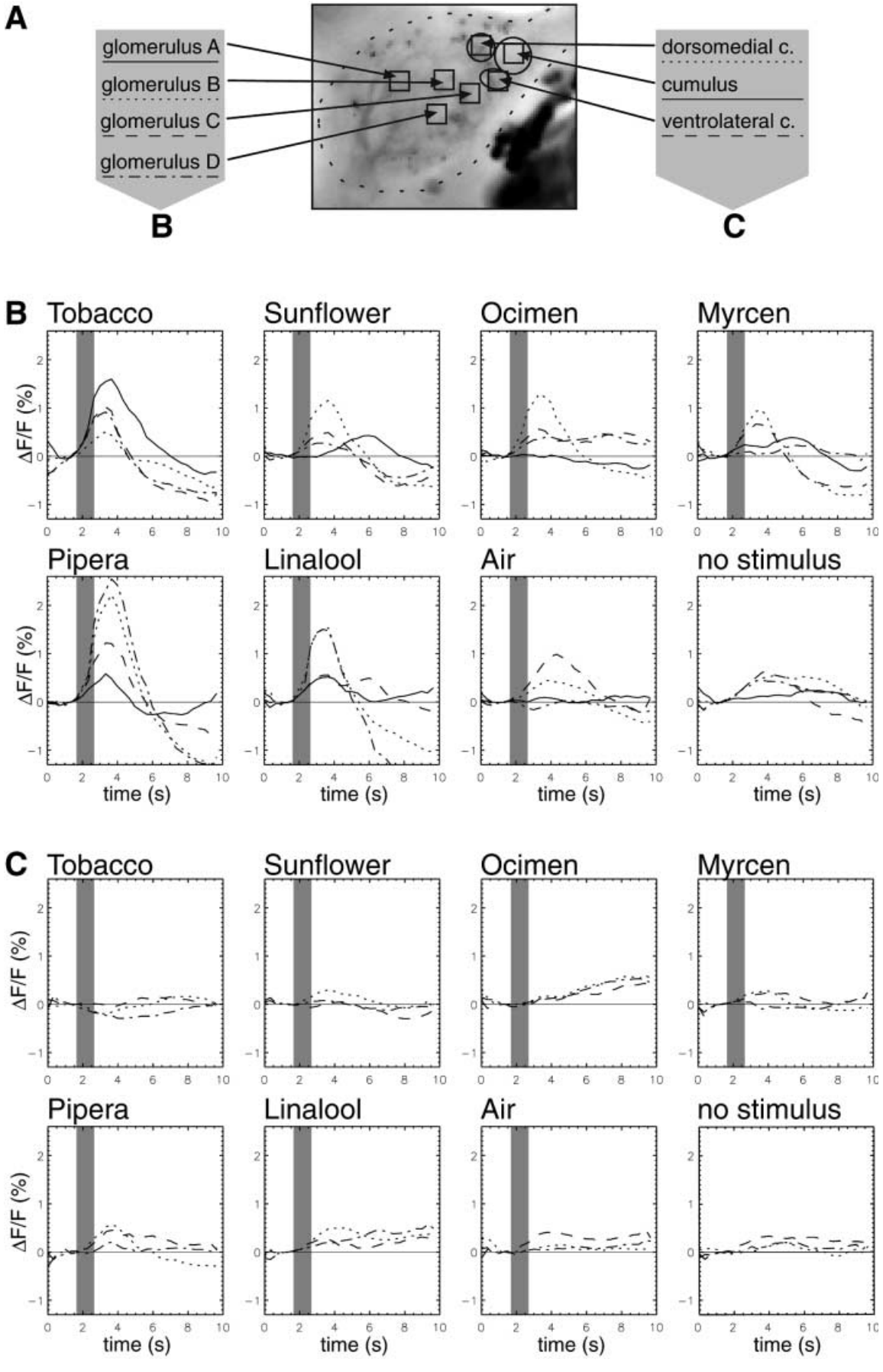
A
glomerulus A
glomerulus B
glomerulus C
glomerulus D
dorsomedial c.
cumulus
ventrolateral c.
B
C
B
Tobacco
Sunflower
Ocimen
Myrcen
Pipera
Linalool
Air
no stimulus
ΔF/F (%)
time (s)
C
Tobacco
Sunflower
Ocimen
Myrcen
Pipera
Linalool
Air
no stimulus
ΔF/F (%)
time (s)

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Fig. 4A–C Glomerular calcium responses to plant-odour stimulation in the antennal lobe of a *H. virescens* male. Each plot shows the time-course of the response in different glomeruli, identified by line style, to a single odour stimulus. Odour concentration is 1 µl for all stimuli. **A** Position of glomeruli evaluated in **B** and **C**. **B** Responses in four "ordinary" glomeruli. **C** Responses in the three visible compartments of the MGC, the dorsomedial, the cumulus, and the ventrolateral compartment. No responses are apparent in the MGC to plant odours

nent. Furthermore, this component differed between stimuli, thus worsening the comparability of the representations. However, visual inspection of the patterns always showed that the patterns of a particular odour were characteristic for that odour.

Female moths: responses to periodic stimulation

In order to investigate the temporal properties of the calcium responses, we applied repeated odour stimulation to the antennal lobe, either at an ISI 10 s or 5 s. Generally, intracellular calcium concentration in the glomeruli followed the 10-s rhythm well. After an initial adaptation which reduced the response sizes to the following stimulations, activity peaks were reliable and well in phase with the stimulus (Fig. 8). Some glomeruli showed "off" responses, i.e. intracellular calcium concentration decreased at stimulus time, and increased after stimulus offset, suggesting that the glomerulus is inhibited during odour presentation. Due to the periodic stimulation, this led to alternating activity in the odour

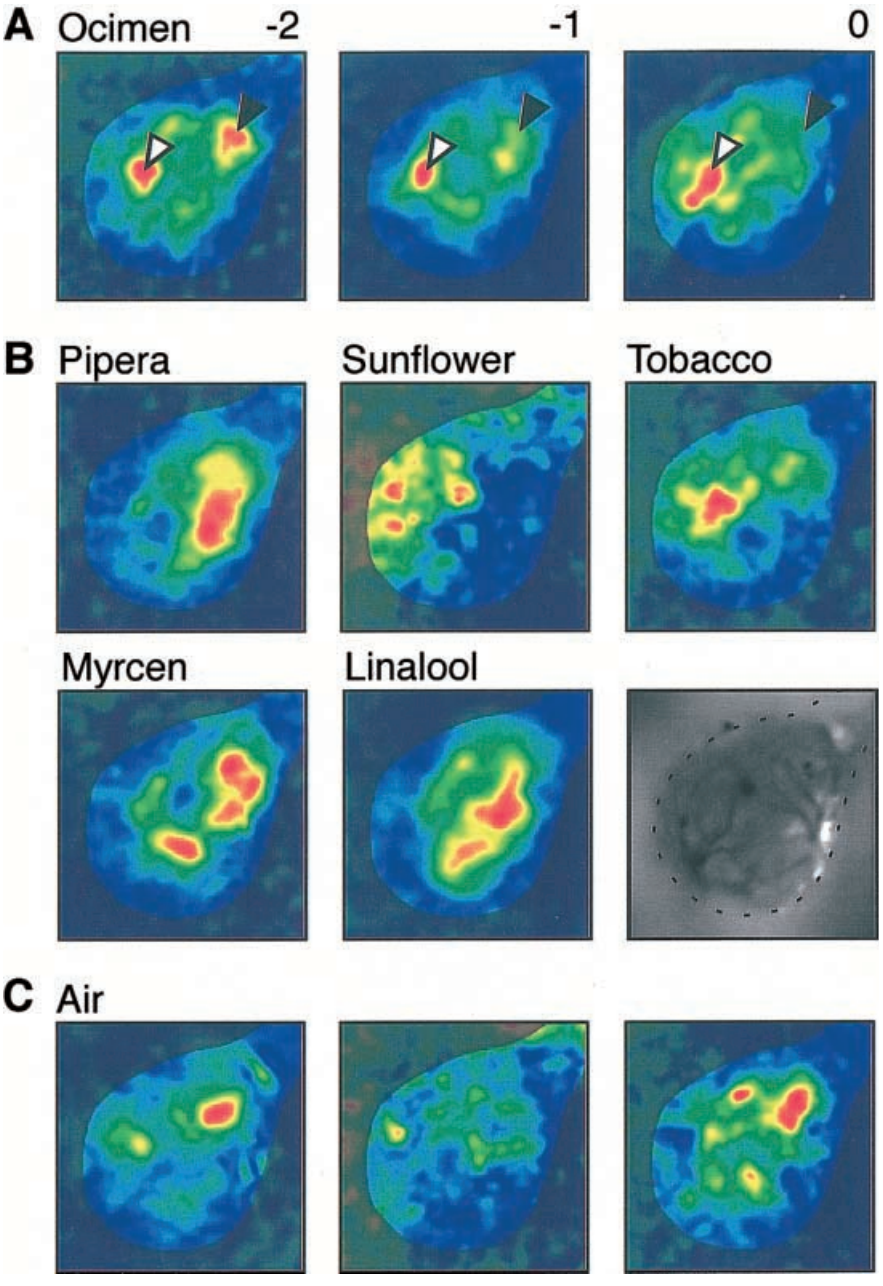

Fig. 5A–C Spatial response patterns to plant odour stimulation in the antennal lobe of a female *H. virescens* moth. The area outside the antennal lobe has been *shaded*. Each image is scaled to its own minimum and maximum. **A** Responses to ocimen at three different concentrations (0.01 µl, 0.1 µl, 1 µl). Note that with one glomerulus increasing its activity with increasing concentration (*open arrowhead*, compare with Fig. 6), another glomerulus active with the same intensity for all three concentrations (*closed arrowhead*) becomes negligible for the pattern: since each image is scaled to its own maximum, the latter glomerulus appears to disappear from the false-colour coded image. **B** Spatial response patterns to five different odours, and anatomical view of the area imaged. **C** Three different responses to the control air stimulation

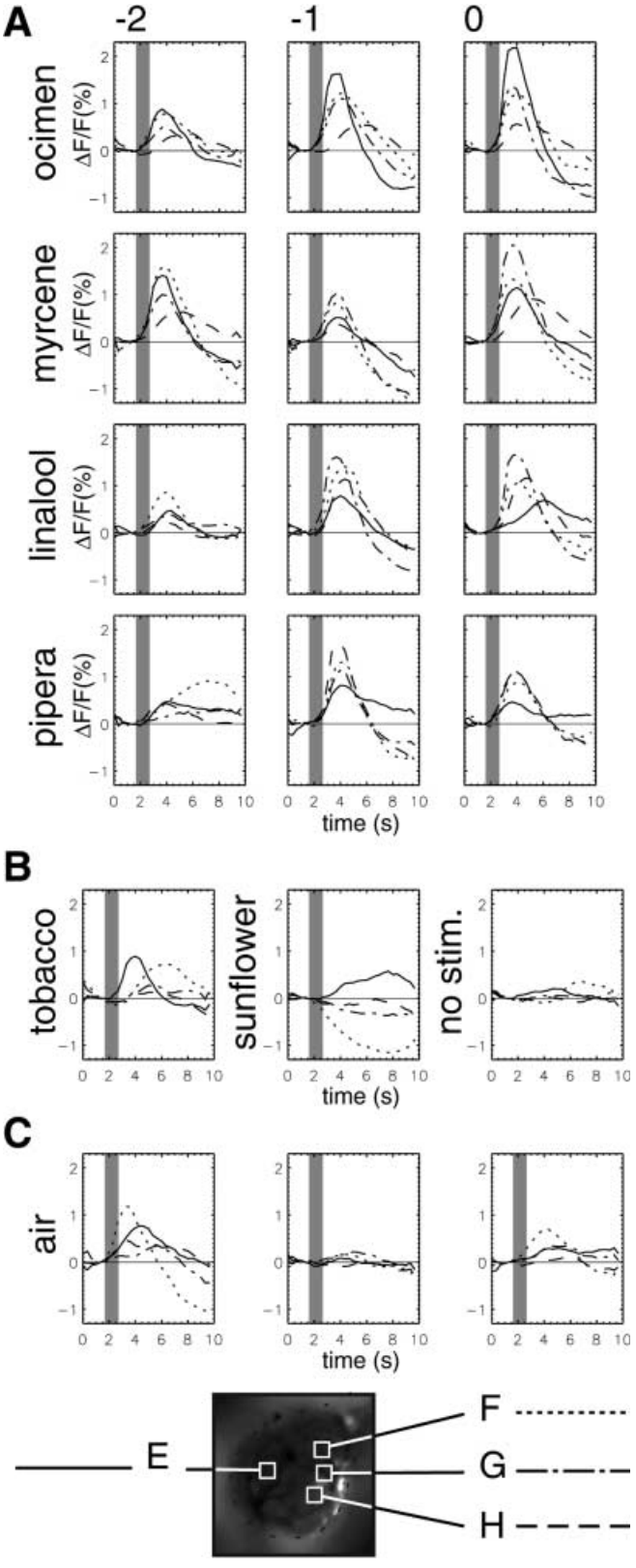

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Fig. 6A–C Glomerular calcium responses to plant-odour stimulation in the antennal lobe of a female *H. virescens* moth. Each plot shows the time-course of the response in different glomeruli to a single odour stimulus. Stimulus is indicated by *grey shading*. **A** For linalool, pipera, myrcene and ocimen responses are shown for three different concentrations (0.01 µl, 0.1 µl, 1 µl). **B** Responses to tobacco, sunflower, and the no-stimulus control are shown. **C** Three different responses to air. The position of the glomeruli taken is shown at the bottom; labelled *E–H*, in order to avoid any confusion with the glomeruli in male moths chosen for Fig. 4. Same animal as in Fig. 5. Note the very strong dose dependency of glomerulus E for ocimen and glomerulus G for myrcene and linalool. Response to pipera is weaker at the highest concentration as compared to the intermediate concentration

responsive and the "off" glomeruli. "Off"-glomeruli were odour specific, i.e. different glomeruli showed "off"-responses for different odours. Other glomeruli, however, lost the timing imposed by the repetitive stimulation, and became random in their activity. This means that only a subgroup of the glomeruli active in the first response also participated in the response to subsequent stimuli. When increasing the stimulation frequency to ISI = 5 s, responses decreased sharply. Interestingly, the most prominent glomeruli in each pattern still followed the stimulation timing, but the intensity was much weaker. Therefore, the false-colour coded spatial pattern appears to be less sharp with ISI = 5 s as compared to ISI = 10 s (Fig. 8) despite the fewer glomeruli following the stimulus timing. Part of the reduction is probably due to adaptation in the receptor cells. However, the antennal lobe network probably causes an additional reduction in response amplitude, since it only appears in some of the stimuli within the sequence. No "off" responses were observed with ISI = 5 s. When reducing stimulus concentration the responses became weaker, and again "off" responses were not observed (Fig. 8F). Note that the spatial pattern calculated from ISI = 5 s with a strong stimulus, and the weaker stimulus concentration at ISI = 10 s lead to very similar spatial patterns (compare Fig. 8D with Fig. 8E).

Responses to mechanosensory stimulation (air-control) were less reliable: stimulus-locked responses and stimulus independent responses could both be observed. We did not find a glomerulus that responded to every air-puff in the sequence (Fig. 8F). These responses were slightly larger than background activity.

Discussion

Responses to pheromones in the male MGC

Optical recording confirmed that within the MGC individual subcompartments deal with processing of single pheromone components. The cumulus, being the largest compartment, specifically responded to the major pheromone component Z11–16:AL (Figs. 1, 2). Indeed, receptor neurones specifically responding to this component have been shown to project to the cumulus (Hansson et al. 1995; Berg et al. 1998). Furthermore, projection neurones with dense branching in the cumulus also preferentially respond to Z11–16:AL (Chris-

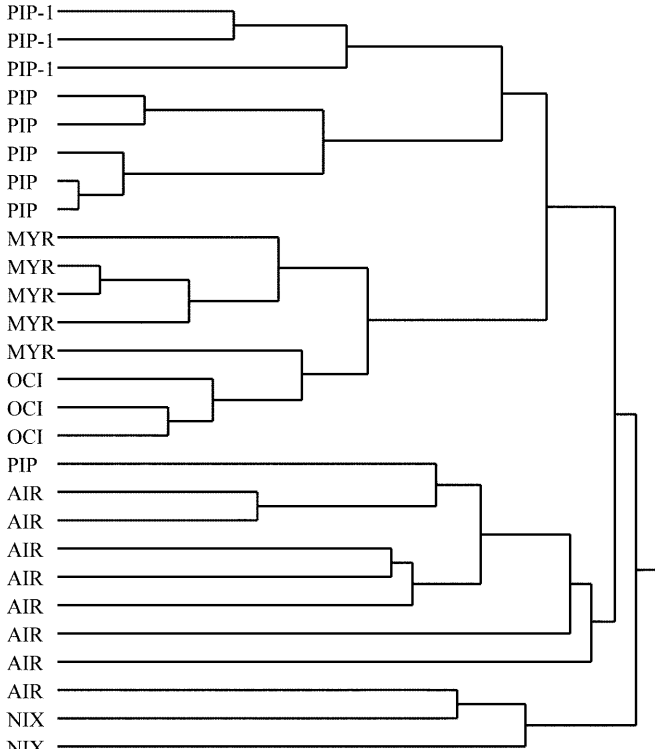

Fig. 7 Cluster analysis of the glomerular responses to stimulation with different odours in a single female moth (different animal from Figs. 5 and 6). Each response pattern was represented as a vector, where each position of the vector corresponds to a glomerulus, and the numerical entry corresponds to the response strength to that odour in that glomerulus. Note that ocimen responses group in a single cluster; myrcene responses group in the neighbouring cluster, with the exception of a single myrcene measurement that joins the ocimen group. Pipera also forms a uniform cluster, which splits into two subclusters, one for high concentrated pipera (1 µl), one for intermediate concentration (0.1 µl). No other concentration series were recorded in this animal

tensen et al. 1995a; Vickers et al. 1998). Similarly, the dorsomedial compartment is selective for the second pheromone component, Z9–14:AL, of *H. virescens,* with respect to its innervation by receptor neurones (Berg et al. 1998) and by projection neurones (Berg et al. 1998; Vickers et al. 1998), and the overall optical response (this paper). The two ventral compartments, the ventrolateral and the ventromedial, are selective for the two interspecific signals Z11–16:OH and Z11–16:AC. In our study, because of the orientation of the antennal lobe, we could only monitor the response to Z11–16:OH in the ventrolateral compartment. The separation of the afferent receptor neurone axons to these two compartments could not be unambiguously determined, because the neurones sensitive to the two components are co-localized in the sensilla, and were always stained together in an electrophysiological tracing study (Berg et al. 1998). Projection neurones responding to the alcohol had arborizations in the ventrolateral, and projection neurones responding to the acetate in the ventromedial compartment (Vickers et al. 1998). We also found that stimulation with high concentrations of Z9–14:AL leads to the ventrolateral compartment being activated, in accordance with that found for receptor neurones selective for Z9–16:OH (Berg et al. 1998).

How are pheromones represented in the MGC of moths? First, from the receptor neurones to the MGC subcompartments, the MGC represents a labelled line system, because each compartment is selective for a specific component. Second, only a comparison across the compartments is behaviourally relevant, because only by comparing the activity would the animal know whether it encountered an intraspecific or an interspecific pheromone plume, thus making it an across-glomeruli code. Therefore, pheromone information is coded in a combinatorial manner within the MGC, with each glomerulus corresponding to one (chemical) information channel.

How independent is this code from activity in the rest of the antennal lobe? We never found activation in the MGC to plant odours (Fig. 4B), and never found responses to pheromone stimulation in the ordinary glomeruli, with exception of mechanosensory responses (Fig. 1A; for a discussion of these responses see below). Therefore, the MGC appears to be an independent, specialized and self-sufficient structure for the pheromone system, as is suggested by decades of olfactory research.

In our measurements, we used – for each pheromone substance – concentrations of 0.2 µg, 2 µg and 20 µg on the filter papers in the cartridges. These concentrations are in the range of those used in tip recordings from trichoid sensilla, but higher than those used for recordings from the sensillum base (Berg et al. 1998). When recording from projection neurones, much lower concentrations can be used (Christensen et al. 1995a). The response in the cumulus to the major component Z11–16:AL increased with increasing concentration (Fig. 3). On the contrary, response to the minor component Z9–14:AL in the dorso-medial compartment decreased with increasing stimulus concentration. This may be due to overloading of the receptors, or to very fast adaptation, which would be more prominent in the minor component since the system is tuned to lower concentrations. The responses to the second stimulus after 1 min corroborate this interpretation: the second response matches the first only for weak concentrations of Z11–16AL – in all other cases the second response is diminished. The response to Z11–16:OH also decreased with increasing concentration, though this effect was less pronounced than with Z9–14:AL. Another explanation may involve the interglomerular connections in the MGC: with increasing concentrations, unspecific responses have been described for receptor neurones that lead to activity in additional glomeruli (see, for example, the response to Z9–14:AL in the ventrolateral compartment, Fig. 2). Inhibitory local interneurones between the active glomeruli may then lead to the paradox concentration effect observed.

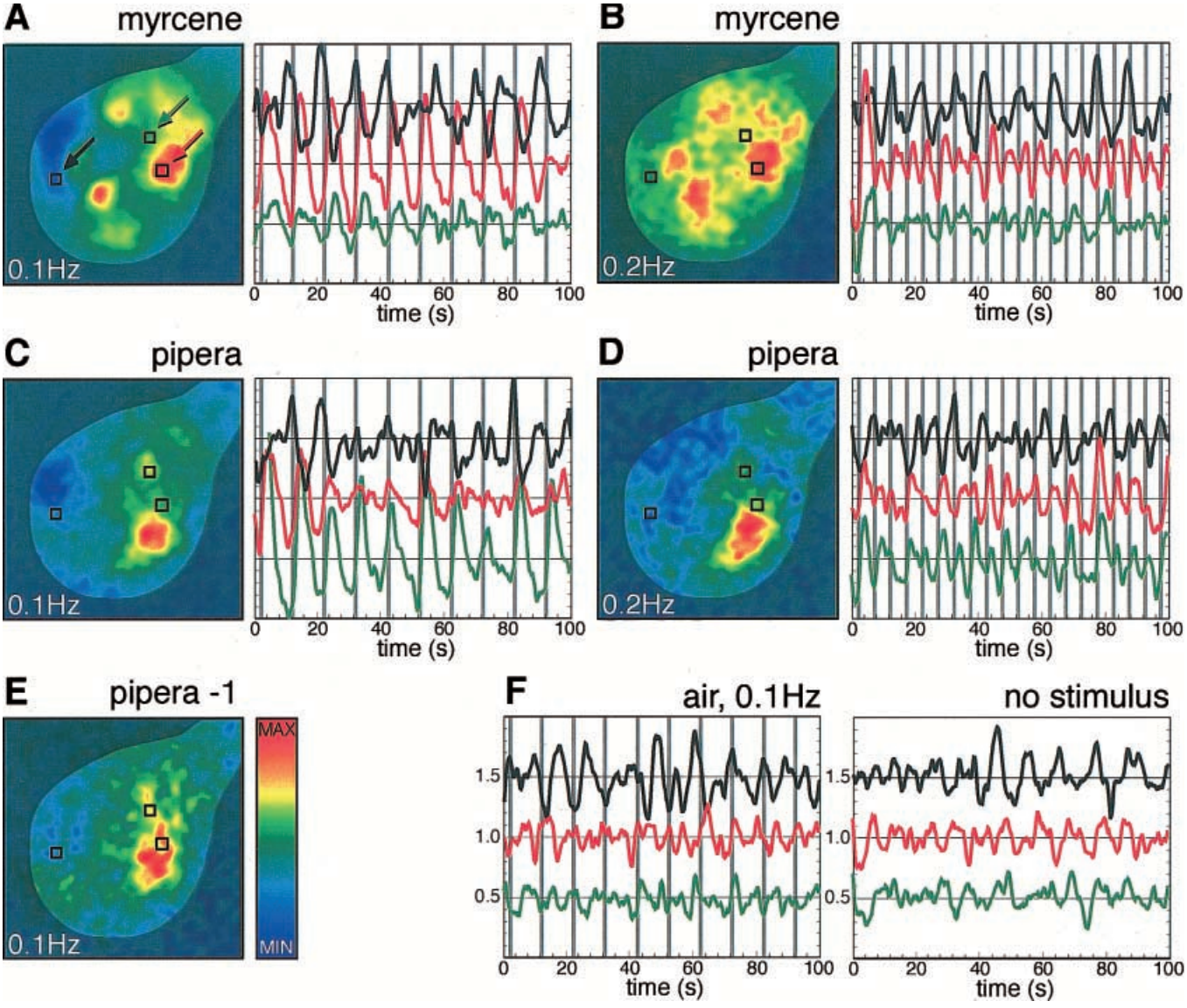

Fig. 8A–F Glomerular calcium responses to periodic plant-odour stimulation in the antennal lobe of a female *H. virescens* moth. Odour pulses were delivered either every 10 s [interstimulus interval (ISI) = 10 s] or every 5 s (ISI = 5 s), and are shown as *vertical grey-shaded bars*. **A** Myrcene, with ISI = 10 s. The false-colour coded image shows several activated glomeruli (red and yellow) and an "off" region (blue, see false-colour bar in **E**). Three glomeruli are marked with *squares*; their time-courses are shown at the right. The red curve follows every stimulus with an excitation, the green curve has weaker and slower responses, but also follows every stimulus, whereas the black curve is inhibited by the stimuli and shows delayed excitatory responses. **B** Spatial pattern and time-courses for myrcene, with ISI = 5. Compared to **A**, responses are weaker, the "off" response is missing, and the spatial pattern is less focalised. The red curve follows every stimulus, the green curve responds only to some of the stimuli, whereas the black ("off") curve shows a random course. **C** Spatial pattern and time-courses for pipera, ISI = 10 s. In the time-courses, the green line follows every stimulus, while red and black are random. **D** Spatial pattern and time-courses for pipera, ISI = 5 s. **E** Spatial pattern for pipera at low concentration (tenfold dilution), ISI = 10. Note that the spatial pattern for the higher frequency stimulation (**D**) and the lower concentration of the odour (**E**) are almost identical; in particular, the "off"-response is missing. **F** Time-courses of the response to air, ISI = 10 s, and for no stimulation. Note that overall activity is higher when stimulating with clean air, but no glomerulus is locked to the stimulus time pattern

Responses to air-puffs and spontaneous activity

When stimulating with the air control we often found significant calcium activity, both in the male and the female antennal lobe (Figs. 1, 3, 4, 5). Both excitatory and inhibitory responses are visible in electrophysiological projection neurone recordings in *Manduca sexta* (Waldrop et al. 1987; Kanzaki et al. 1989) and *H. virescens* (B. Berg, personal communication). The most stunning property of these responses is that they are not reliable, and vary in size and position for different measurements. This makes it impossible to use the air control as a blank, which is why we had to develop a different method in order to correct for bleaching. The reason for the lack in predictability is most likely due to the complexities of aerodynamic turbulences. Stimulation occurred by switching between two adjacent cartridges; therefore, the source direction of the air changed along with the odour, and this leads to unpredictable eddies. Furthermore, the antennae were moving freely,

and therefore possibly at slightly different positions for different stimuli. In a subset of experiments, the odour was delivered into the continuous air stream. This arrangement strongly reduces the turbulence differences between stimuli. Indeed, in these experiments responses to air were much more consistent between trials. However, the small increase in airflow pressure caused by the stimulus air being added to the constant air stream was still sufficient to elicit clear calcium signals. In optical recording studies of honeybees where stimulation also had a mechanosensory component (by adding the stimulus into a constant air stream), no response was found to the mechanical component (Galizia et al. 1999a; Sachse et al. 1999). In the honeybee the main component of the signal originates from receptor cells, with only a minor part due to interneurones and projection neurones (Galizia et al. 1998). Both the higher spontaneous activity and the responses to air-puffs found in moths indicate that a higher proportion of interneurones and projection neurones contribute to the signals in this species. The "off" responses found when repeatedly stimulating with an odour further support this view.

Responses to plant odours in the male and female antennal lobe of *H. virescens*

Plant odours elicited distributed activity patterns in the ordinary glomeruli in males, or in the antennal lobe of female moths. That is, each odour elicited activity in several glomeruli, and individual glomeruli took part in the activity patterns of different odours. Within insects, such an organization of non-pheromonal odour responses has been shown with radioactive 2-deoxyglucose staining in flies (Rodrigues 1988), and using optical imaging in honeybees (Joerges et al. 1997; Galizia et al. 1998) and ants (Galizia et al. 1999b). When comparing the activity patterns between different individuals, it is crucial to map the responses onto morphologically identified glomeruli, because the view of the antennal lobe is not identical in different preparations (Galizia et al. 1999a; Sachse et al. 1999). This was not possible in this study, because no morphological atlas of ordinary glomeruli is available for *H. virescens*; therefore, we did not attempt to compare the elicited patterns between individuals.

Only few electrophysiological data are available for projection neurone responses to plant odours in moths. More data have been published about single-cell recordings of receptor cells. Often receptor neurones respond with high selectivity to only a few compounds if stimulated with biologically relevant volatiles at low concentrations, as demonstrated by linked gas chromatography-electrophysiology (Tømmerås and Mustaparta 1987; Blight et al. 1995; Wibe and Mustaparta 1996; Røstelien et al. 2000a, b). In our imaging studies we would therefore expect that – with decreasing odour concentration – the across-glomeruli patterns would narrow down to a single glomerulus being activated. As a result of the superposition of the mechanosensory and the olfactory stimulus in our experiments, this could not be observed. For example, in Fig. 5A, the response to low ocimen concentrations consists of two glomeruli (open and closed arrowheads). With increasing odour concentration, the pattern appears to sharpen rather than to broaden, because the ocimen-responsive glomerulus increases its activity (open arrowhead, and continuous line in Fig. 6A), while the air-responsive glomerulus is concentration independent (closed arrowhead, and dotted line in Fig. 6A).

Receptor neurone recordings have shown a subpopulation of receptor cells selective for trans-β-ocimen and β-myrcene (Røstelien et al. 2000b). Indeed, we found their representations to be more similar to each other than each of them is to pipera, as evidenced by a cluster analysis of the evoked patterns (Fig. 7). This analysis also showed that different concentrations of pipera are grouped within one cluster.

Responses to different odour concentrations suggest that inter-glomerular inhibition is a strong factor in the olfactory code, in accordance with electrophysiological findings (Christensen et al. 1996). Glomeruli differed in the concentration dependency of their responses to different odours: for example, glomerulus E was the strongest and concentration dependent for ocimen, but it was the strongest for myrcene only at low concentrations, whereas glomerulus G increased its response to myrcene with increasing concentration. Furthermore, glomerulus H, which was immediately activated by pipera, was also activated by the other odours, but responded with a delay of up to 2 s. These findings still have to be corroborated by electrophysiological recordings from interneurones and projection neurones in ordinary glomeruli of moths. In honeybees, when stimulating with odour mixtures, inhibitory effects were found in the optical responses of olfactory glomeruli (Joerges et al. 1997). In our study, stimulating with high concentrations of pipera led to a decrease rather than an increase of response intensity (Fig. 6A). Pipera is a complex mixture of many substances, and evokes a widespread activity in the antennal lobe (Fig. 5B). The response reduction at high concentrations is probably the effect of inter-glomerular inhibition.

Responses to repeated stimulations

Pheromone-sensitive projection neurones are able to follow pulsed stimulation up to a frequency of 10 Hz (Almaas et al. 1991; Lei and Hansson 1999). In our calcium measurements of plant odour responses, an ISI of 0.2 Hz already led to a significant deterioration of the signal. This deterioration is not due to calcium buffering, because it only affected some glomeruli, while others could still follow at ISI of 0.2 Hz. Possibly, the pheromone system is tuned to much higher pulse frequencies than the plant-odour system. Part of the response

reduction is certainly due to adaptation. However, most of the reduction must be due to network properties in the antennal lobe, since occasionally individual responses within the pulse train were very strong (Fig. 8). Note that some glomeruli followed the pulsating stimulus well, while others lost their time grip. Consequently, the glomerular response pattern to the odour is not identical for successive stimuli in a sequence. The question arises, therefore, whether a sub-pattern of the observed calcium response is sufficient to be behaviourally active, or whether the spatial pattern encodes both odour quality and some information about odour-puff sequences. It is interesting to note that the more reliable glomeruli are those that still respond to the lower odour concentration (compare Fig. 8D with Fig. 8E).

Some glomeruli showed "off" responses specific to the tested odours. These responses were visible at ISI = 10 s, but disappeared when higher pulse frequencies were applied (ISI = 5 s) or when the odour concentration was reduced (Fig. 8). The inhibitory cells are driven by the excitatory input from the olfactory receptor neurons. Increased odour concentration leads to an increase in afferent input, which probably enhances the effect of the inhibitory network, explaining why "off" responses were only visible at higher concentrations. Increased hyperpolarisation with increasing odour concentration has been reported from mitral cells in the salamander OB (Hamilton and Kauer 1989). "off"-responses have been reported from projection neurones in honeybees (Sun et al. 1993) and moths (Kanzaki et al. 1989), and arise from the inhibitory network in the antennal lobe (Waldrop et al. 1987). The finding that the "off"-glomeruli differ for different odours may indicate that inhibitory interconnections are glomerulus specific, so that a particular odour will only lead to inhibitory input in selected glomeruli. Individual local interneurones only innervate parts of the antennal lobe, and not all glomeruli. When we increased stimulus frequency the "off" responses were reduced. This could have two (non-exclusive) explanations. First, "off" responses consisted of a slight reduction in the signal when the stimulus was presented, and a delayed excitatory response. With ISI = 5 s, the excitatory response was coincident with the next stimulus, thus leading to a mutual annihilation of the effects. Second, increasing the stimulus frequency led to weaker responses, probably due to stronger adaptation (see above); thus, the pattern resulting from higher frequency stimulation is comparable to the response to reduced odour concentration, which also lacks "off" responses.

Concluding remarks

In this study, we have confirmed electrophysiological data about the organization of the MGC. We have shown that plant odours are encoded as across-glomeruli patterns in the 'ordinary' glomeruli, and that these glomeruli differ in their ability to follow pulsed stimuli as a function of the odour used. Furthermore, mechanosensory stimulation also elicits glomerular responses. More studies are needed to map the responses to identified glomeruli, and electrophysiological and imaging studies should be combined, in order to understand the spatial and temporal structures of olfactory coding at the level of antennal lobe glomeruli.

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References

Almaas TJ, Christensen TA, Mustaparta H (1991) Chemical communication in heliothine moths. I. Antennal receptor neurons encode several features of intra- and interspecific odorants in the corn earworm moth *Helicoverpa zea*. J Comp Physiol A 169: 249–258

Berg BG, Almaas TJ, Bjaalie JG, Mustaparta H (1998) The macroglomerular complex of the antennal lobe in the tobacco budworm moth *Heliothis virescens*: specified subdivision in four compartments according to information about biologically significant compounds. J Comp Physiol A 183: 669–682

Berg BG, Tumlinson JH, Mustaparta H (1995) Chemical communication in heliothine moths. 4. Receptor neuron responses to pheromone compounds and formate analogues in the male tobacco budworm moth *Heliothis virescens*. J Comp Physiol A 177: 527–534

Blight MM, Pickett JA, Wadhams LJ, Woodcock CM (1995) Antennal perception of oilseed rape, *Brassica napus* (Brassicacea), volatiles by the cabbage seed weevil *Ceuthorynchus assimilis* (Coeoptera:Curcullionidae). J Chem Ecol 21: 1649–1664

Boeckh J, Boeckh V (1979) Threshold and odor specificity of pheromone-sensitive neurons in the deuticerebrum of *Antheraea pernyi* and *A. polyphemus* (Saturnidae). J Comp Physiol A 132: 235–242

Christensen TA, Hildebrand JG (1987) Male-specific, sex pheromone-selective projection neurons in the antennal lobes of the moth *Manduca sexta*. J Comp Physiol A 160: 553–569

Christensen TA, Mustaparta H, Hildebrand JG (1989) Discrimination of sex pheromone blends in the olfactory system of the moth. Chem Senses 14: 463–477

Christensen TA, Mustaparta H, Hildebrand JG (1991) Chemical communication in heliothine moths. II. Central processing of intra- and interspecific olfactory messages in the male corn earworm moth *Helicoverpa zea*. J Comp Physiol A 169: 259–274

Christensen TA, Mustaparta H, Hildebrand JG (1995a) Chemical communication in heliothine moths. 4. Parallel pathways for information processing in the macroglomerular complex of the male tobacco budworm moth *Heliothis virescens*. J Comp Physiol A 177: 545–557

Christensen TA, Harrow ID, Cuzzocrea C, Randolph PW, Hildebrand JG (1995b) Distinct projections of two populations of olfactory receptor axons in the antennal lobe of the sphinx moth *Manduca sexta*. Chem Senses 20: 313–323

Christensen TA, Heinbockel T, Hildebrand JG (1996) Olfactory information processing in the brain: encoding chemical and temporal features of odors. J Neurobiol 30: 82–91

Galizia CG, Nägler K, Hölldobler B, Menzel R (1998) Odour coding is bilaterally symmetrical in the antennal lobes of honeybees (*Apis mellifera*). Eur J Neurosci 10: 2964–2974

Galizia CG, Sachse S, Rappert A, Menzel R (1999a) The glomerular code for odor representation is species-specific in the honeybee *Apis mellifera*. Nat Neurosci 2: 473–478

Galizia CG, Menzel R, Hölldobler B (1999b) Optical imaging of odour-evoked glomerular activity patterns in the antennal lobes of the ant *Camponotus rufipes*. Naturwissenschaften 86: 533–537

Hamilton KA, Kauer JS (1989) Patterns of intracellular potentials in salamander mitral/tufted cells in response to odor stimulation. J Neurophysiol 62: 609–625

Hansson BS, Christensen TA, Hildebrand JG (1991) Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. J Comp Neurol 312: 264–278

Hansson BS, Ljungberg H, Hallberg E, Löfstedt C (1992) Functional specialization of olfactory glomeruli in a moth. Science 256: 1313–1315

Hansson BS, Almaas TJ, Anton S (1995) Chemical communication in heliothine moths. 5. Antennal lobe projection patterns of pheromone detecting olfactory receptor neurons in the male *Heliothis virescens* (Lepidoptera: Noctuidae). J Comp Physiol A 177: 535–543

Hildebrand JG, Shepherd GM (1997) Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. Annu Rev Neurosci 20: 595–631

Homberg U, Christensen TA, Hildebrand JG (1989) Structure and function of the deutocerebrum in insects. Annu Rev Entomol 34: 477–501

Joerges J, Küttner A, Galizia CG, Menzel R (1997) Representations of odours and odour mixtures visualized in the honeybee brain. Nature (Lond) 387: 285–288

Kanzaki R, Arbas EA, Strausfeld NJ, Hildebrand JG (1989) Physiology and morphology of projection neurons in the antennal lobe of the male moth *Manduca sexta*. J Comp Physiol A 165: 427–453

King JR, Christensen TA, Hildebrand JG (2000) Response characteristics of an identified, sexually dimorphic olfactory glomerulus. J Neurosci 20: 2391–2399

Koontz MA, Schneider D (1987) Sexual dimorphism in neuronal projections from the antenna of silk moths (*Bombyx mori, Antherea polyphemus*) and the gypsy moth (*Lymantria dispar*). Cell Tissue Res 249: 39–59

Lei H, Hansson BS (1999) Central processing of pulsed pheromone signals by antennal lobe neurons in the male moth *Agrotis segetum*. J Neurophysiol 81: 1113–1122

Masson C, Mustaparta H (1990) Chemical information processing in the olfactory system of insects. Part 2: central nervous system, development, and plasticity. Physiol Rev 70: 215–245

Rodrigues V (1988) Spatial coding of olfactory information in the antennal lobe of *Drosophila melanogaster*. Brain Res 453: 299–307

Røstelien T, Borg-Karlson AK, Fäldt J, Jacobsen U, Mustaparta H (2000a) The plant sesquiterpene germacrene D specifically activates a major type of antennal receptor neurons of the tobacco budworm moth *Heliothis virescens*. Chem Senses 25: 141–148

Røstelien T, Borg-Karlson AK, Mustaparta H (2000b) Selective receptor neuron responses to *E*-ocimene and -myrcene, *E*,*E*-farnesene or to related homo-terpenes in *Heliothis virescens*, identified by gas chromatography linked to electrophysiology. J Comp Physiol A 186: 833–847. DOI 10.1007/s003590000136

Sachse S, Rappert A, Galizia CG (1999) The spatial representation of chemical structures in the antennal lobe of honeybees: steps towards the olfactory code. Eur J Neurosci 11: 3970–3982

Sun X-J, Fonta C, Masson C (1993) Odour quality processing by bee antennal lobe interneurones. Chem Senses 18: 355–377

Tømmerås BÅ, Mustaparta H (1987) Chemoreception of host volatiles in the bark beetle *Ips typographus*. J Comp Physiol A 161: 705–710

Vickers NJ, Christensen TA, Hildebrand JG (1998) Combinatorial odor discrimination in the brain: attractive and antagonist odor blends are represented in distinct combinations of uniquely identifiable glomeruli. J Comp Neurol 400: 35–56

Waldrop B, Christensen TA, Hildebrand JG (1987) GABA-mediated synaptic inhibition of projection neurons in the antennal lobes of the sphinx moth, *Manduca sexta*. J Comp Physiol A 161: 23–32

Wibe A, Mustaparta H (1996) Encoding of plant odors by receptor neurons in the pine weevil (*Hylobius abietis*) studied by linked gas chromatography electrophysiology. J Comp Physiol A 197: 331–334