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Dose–Response Characteristics of Glomerular Activity in the Moth Antennal Lobe

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Abstract

Odours are represented as unique combinations of activated glomeruli in the antennal lobes of insects. Receptor neurons arborizing in the glomeruli are not only qualitatively selective, but in addition respond to variations in stimulus concentration. As each glomerulus likely represents a single receptor neuron type, optical recordings of calcium changes in insect antennal lobes show how concentration variations affect a large population of afferents. We measured the glomerular responses in the moth *Spodoptera littoralis* to different concentrations of plant-related odorants. Localized calcium responses were shown to correspond to individual glomeruli. We found that the dynamic range of glomerular responses spanned 3–4 log units of concentration and the most strongly responding glomeruli often reached a plateau at high stimulus doses. Further, we showed that the single most active glomerulus was often not the same across concentrations. However, if the principal glomerulus moved, it was generally to an adjacent or proximal glomerulus. As concentration increased, a higher number of glomeruli became activated. Correlations of glomerular representations of the same compound at different doses decreased as the difference in concentration increased. Moreover, representations evoked by different odorants were more correlated at high than at low doses, which means that the uniqueness of activity patterns decreased with increasing concentration. Thus, if odours are coded as spatial patterns of glomerular activity, as has been suggested, these olfactory codes are not persistent across concentrations.

Key words: olfaction, *Spodoptera littoralis*, optical imaging, plant volatile, spatial coding

Introduction

The insect olfactory system is not only designed to detect and to discriminate between a vast array of odour molecules, but also to sense changes in stimulus concentration. The molecular density in the environment may vary considerably. For example, low concentrations of flower odours can attract an insect over a long distance. The same insect should also be able to cope with the high concentrations of molecules within the petals of a flower when feeding on nectar, without adapting the system. Olfactory receptor neurons (ORN) respond to increased concentrations by an increase in action potential frequency. A number of studies have shown that insect ORNs respond to molecular concentration changes over several orders of magnitude (Hansson, 1995; Todd and Baker, 1999).

In the antennal lobes (AL) of insects or olfactory bulbs of vertebrates, odours are represented by different combinations of responding glomeruli (Sharp *et al.*, 1975; Kauer, 1988; Rodrigues, 1988; Cinelli *et al.*, 1995; Friedrich and Korsching, 1997; Joerges *et al.*, 1997; Distler *et al.*, 1998; Johnson *et al.*, 1998; Galizia *et al.*, 1999; Rubin and Katz, 1999; Uchida *et al.*, 2000; Meister and Bonhoeffer, 2001; Carlsson *et al.*, 2002). Only few studies, however, have paid attention to how concentration variations affect the odour-evoked representations in the lobe or bulb. If a glomerular activity pattern represents a spatial olfactory code, as has been suggested (Johnson *et al.*, 1998; Galizia *et al.*, 1999), relative patterns should remain identical at different concentrations to preserve the qualitative information. If patterns are not identical, quality perception may vary with concentration. Spatial coding, however, likely functions either in parallel or in combination with an olfactory temporal code (Laurent *et al.*, 2001). Temporal characteristics of output signals in moth ALs are not only stimulus-specific, but also vary with concentration (Christensen *et al.*, 2000). The perception of odour quality has in several instances been shown to be concentration-dependent. For example, olfactory responses of the fruit fly *Drosophila melanogaster* shift from attraction to repulsion as the concentration increases (Siddiqi, 1983; Stensmyr *et al.*, 2003). Furthermore, a few psychophysical studies in humans have reported

altered qualitative perception of some odours due to changed concentrations (Gross-Isserof and Lancet, 1988; Arctander, 1994). Glomerular activity patterns in vertebrates, observed in functional imaging experiments, have proven concentration-dependent, often with a recruitment of active glomeruli (Rubin and Katz, 1999; Johnson and Leon, 2000; Fuss and Korsching, 2001; Meister and Bonhoeffer, 2001; Wachowiak and Cohen, 2001; Fried *et al.*, 2002). In the zebra fish, for example, dissimilarity between pairs of activity patterns, evoked by closely related amino acids, was heavily reduced at high concentrations (Fuss and Korsching, 2001). Concentration-invariant activity patterns have, however, also been reported (Joerges *et al.*, 1997; Johnson and Leon, 2000; Wachowiak *et al.*, 2000, 2002).

In a previous Ca^{2+}-imaging study in the moth *Spodoptera littoralis*, we demonstrated that different odorants were represented as unique combinations of activated glomeruli (Carlsson *et al.*, 2002). The questions we aim to address in the present study concern the effect of stimulus concentration on odour representations. We investigate the persistence of glomerular activity across a range of concentrations with respect to location of activity focus, number of activated glomeruli and correlation of patterns at different concentrations. Another imaging study in the moth *Heliothis virescens* indicated that concentration dependency differed between glomeruli (Galizia *et al.*, 2000). Our study extends these data by using a larger range of concentrations. Furthermore, by correlating calcium responses to individual glomeruli we were able to study qualitative pattern changes in detail.

Materials and methods

Animals

Male and female cotton leaf worm moths (*S. littoralis*, 1–5 days post-emergence) were used in the study. The animals have been reared for several generations on a potato-based diet (Hinks and Byers, 1976). The culture has been supplemented with wild-collected insects yearly for the last 7 years. The pupae were separated according to sex and kept in plastic boxes at 70% relative humidity, 23°C and a 16 h/8 h light/dark cycle. Adult moths were supplied with water *ad libitum* until the start of the experiment.

Preparation of animals and optical recordings

Preparation of animals and optical recordings were performed as described elsewhere (Carlsson *et al.*, 2002). Briefly, a calcium-sensitive dye (CaGR-2-AM; Molecular Probes, Eugene, OR) was bath applied to the uncovered brain. After incubation and washing, recordings were carried out *in vivo*.

We used a TILL Photonics imaging system (Gräfelfing, Germany). Filter settings were dichroic: 500 nm; emission LP 515 nm and the preparation was illuminated at 475 nm. Sequences of 40 frames (4 Hz, 200 ms exposure) were recorded through a 20× (NA 0.50; Olympus, Hamburg, Germany) air objective. Stimulation started at frame 12 and lasted 1 s. Images were binned 2× on chip (to 320 × 240 pixels) to increase signal-to-noise ratio. Execution of protocols and initial analyses of data were made using the software Till-vision (TILL Photonics).

A moistened and charcoal-filtered continuous air stream (30 ml/s) ventilated the antenna ipsilateral to the recorded AL through a glass tube (7 mm internal diameter). The glass tube ended ~10 mm from the antenna. An empty Pasteur pipette was inserted through a small hole in the glass tube, blowing an air stream of ~15 ml/s. Air was blown (~15 ml/s) through the odour-laden pipette by a manually triggered puffer device (Syntech, Hilversum, The Netherlands) for 1 s into the continuous stream of air. During stimulation the air stream was switched from the empty pipette to the odour laden one, thereby minimizing mechanical influences. Odorants used in the experiment were 1-octanol, geraniol, (±)-linalool and phenylacetaldehyde (PAA). These odorants are biologically relevant to the animal as common components of green leaves and flowers. The purity of the compounds was between 95 and 99%. Odorants were dissolved in paraffin oil and diluted in decadic steps (100 µg/µl–10 ng/µl). Ten microlitres of diluted odorants were applied on filter papers (5 × 15 mm) in doses from 100 ng to 1 mg. The filter papers were inserted in Pasteur pipettes, that were then sealed with Parafilm (American National Can, Chicago, IL) and stored in a freezer until start of experiment. Control stimuli consisted of filter paper with solvent only. Every fifth stimulation was made with a control.

Data evaluation

Square regions of interest (10 × 10 pixels) were drawn within the centre of each glomerulus seen in the anatomical post-stainings (see below). The size of the square (~10 × 10 µm) was well within the boundaries of glomeruli (~50–70 µm diameter), thus minimizing fluorescence overspill from neighbouring glomeruli. Background fluorescence (F) was defined as an average of frames 2–11, i.e. before onset of stimulation. F was subtracted from all frames to yield a dF and signals were expressed as a relative change in fluorescence (dF/F) in the regions of interest. A sequence with control (filter paper with solvent) stimulation was first expressed as relative change in fluorescence (dF/F) and then subtracted from a sequence with odour stimulation in order to correct for bleaching and possible solvent effects. We defined the activity of a glomerulus as the average pixel value of the mean net activity (dF/F odour stimulation – dF/F solvent control) during frames 16–22 (peak of activity).

For correlation analysis we defined a response to an odorant as a multidimensional space where the net activity in each observed glomerulus represents one dimension. The responses were normalized so that the most strongly activated glomerulus for each stimulation was given the value 1.

The response profiles for each odorant and concentration were then compared to each other using the Spearman rank correlation test (JMP 4.02; SAS, Cary, NC). A perfect match between stimuli would yield a value of 1 and perfectly complementary responses would yield a value of –1.

Anatomical staining

In order to visualize the glomeruli, which were not visible in the Ca^{2+}-recordings, we stained the animals with a membrane-bound dye subsequent to the recordings (Figure 1). We used the voltage-sensitive dye RH 795 (Molecular Probes), which has previously been used to stain honeybee glomeruli (Galizia *et al.*, 1999). Outlines were drawn at the glomerular borders and transferred to the sequences of raw data in order to make calculations of activity from individual glomeruli. Certain landmarks, such as remains of tracheae or the borders of the AL, were used to facilitate the alignment of the anatomical images with the physiological images.

Results

In eight females, in which all odorants were tested at all concentrations, we managed to anatomically stain the AL after the recordings. Outlines of visible glomeruli were superimposed and aligned with the false-colour-coded images of odour-evoked responses (Figure 1). Certain landmarks, such as tracheae, were visible both in the calcium recordings and in the post-stainings, which facilitated alignment. The number of visible glomeruli we could observe was between 12 and 20 (mean ± SD = 16 ± 3). In all analyses we first superimposed the glomerular outlines on the sequences of optically recorded calcium activity and all subsequent measurements were made within the boundaries of glomeruli.

Focus of activity

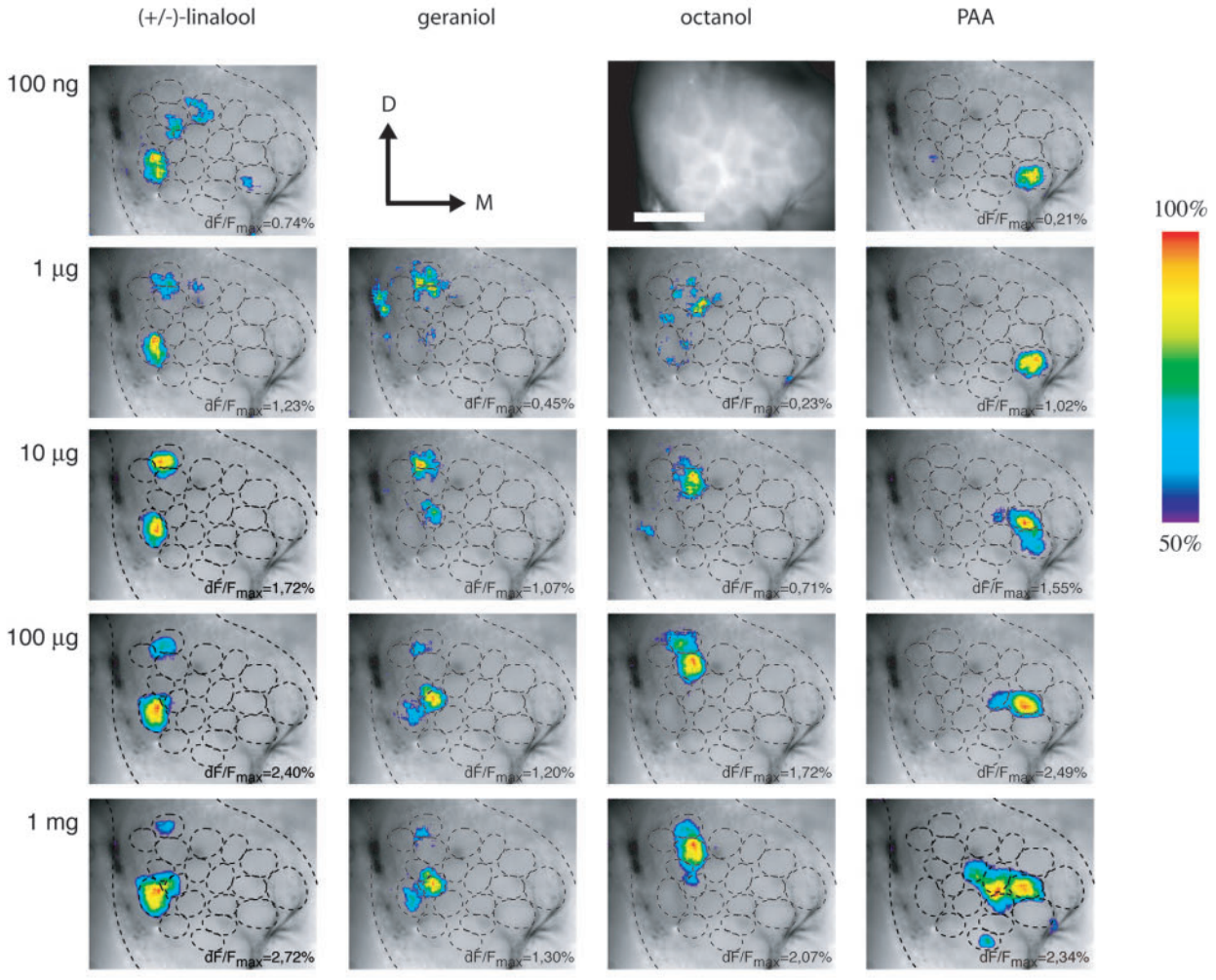
A primary question was whether the focus of activity or the principal glomerulus—i.e. the single glomerulus showing the strongest odour-evoked [Ca^{2+}] increase—persisted across concentrations. Figure 1 shows the response patterns in a single animal to (±)-linalool, geraniol, octanol and PAA at five concentrations (100 ng–1 mg, in decadic steps) expressed as the relative change in fluorescence (d*F*/*F*). Each image is false-colour coded, scaled to the upper 50% of its intensity range and superimposed on grey-scale images from the respective measurement. The threshold, set to ≥50% of the maximal response, excludes low-intensity activity and noise, but emphasizes the location of the principal glomeruli. The three alcohols activated overlapping subsets of glomeruli in the lateral region of the lobe, whereas PAA activated glomeruli in the medial region. Octanol and geraniol did not elicit any detectable response at the lowest concentration (100 ng). The single most activated glomerulus was often different at different concentrations (e.g. PAA and geraniol; Figure 1). Activity foci were only persistent across concentrations in 25% of the recorded concentration series (8 of 32, four odours and eight animals; Table 1). However, movement of activity focus was generally restricted to adjacent or proximal glomeruli. The movement of the principal glomeruli for each of the compounds geraniol, linalool and octanol occurred to neighbouring glomeruli, also highly activated by the other alcohols. These glomeruli were all located in the lateral region of the AL. The principal glomerulus for PAA moved exclusively to neighbouring glomeruli in the medial region of the AL.

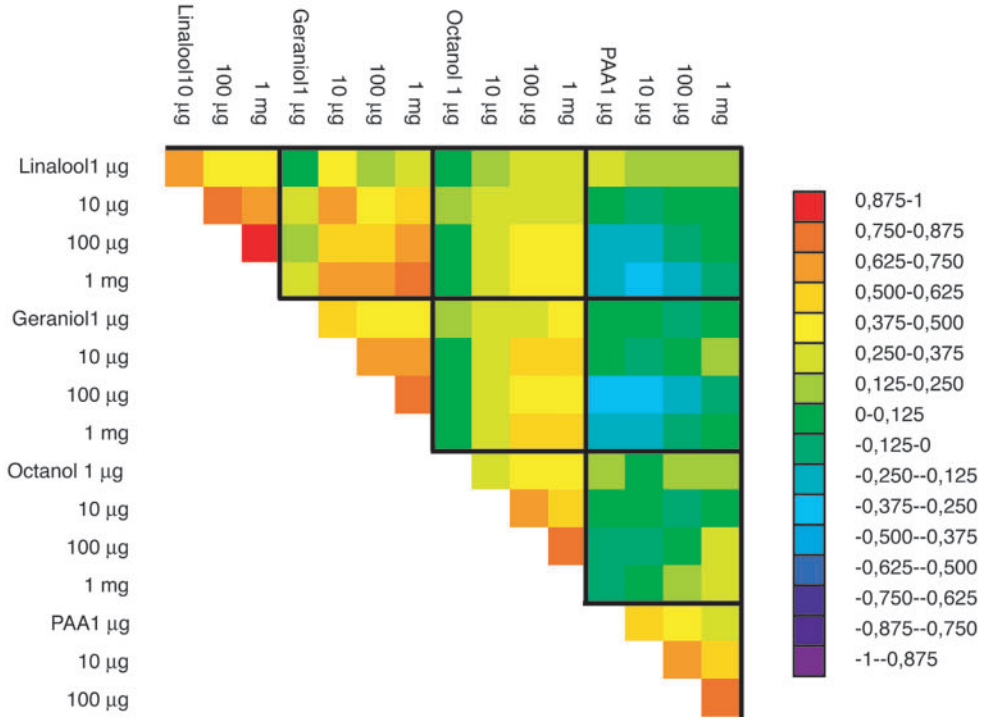
Dynamic range

In Figure 2, we show an example of dose–response curves for all glomeruli in a single animal (same as in Figure 1). In addition, we identified four glomeruli by their position and response at 100 µg (Carlsson *et al.*, 2002). The linalool-, geraniol- and octanol-type glomeruli were in all individuals located in a ventro-dorsal row in the lateral part of the lobe, with the linalool type located most ventrally and the octanol type most dorsally. The PAA-type glomerulus was located in the medial part of the AL. These glomeruli correspond with glomeruli 1, 8, 4 and 16 in Figure 2, respectively. For the identified glomeruli, we calculated the mean normalized responses across animals and concentrations (Figure 3). The most strongly excited glomeruli responded with increasing intensity over 3–4 log units of concentration. Often, the responses to the key compounds reached a plateau, suggesting a sigmoid function. Other glomeruli showed a higher threshold and never reached saturation with the stimulus

Figure 1 **(Top)** Responses to five concentrations of (±)-linalool, geraniol, octanol and phenylacetaldehyde (PAA) in a single female individual. The images show the relative change in fluorescence (d*F*/*F*) and are false-colour coded and thresholded at 50% of the intensity range of each image and superimposed on grey-scale images from the respective measurement. Geraniol and octanol did not elicit any detectable response at the lowest concentration (100 ng) in this animal. Outlines of the lobe and glomeruli, which were visualized in a subsequent anatomical staining (grey-scale inset), have been superimposed on the response images. Construction of the glomerular outlines was made from images of stained glomeruli at several focal depths. Alignment with the false-colour-coded images was facilitated by certain landmarks, such as tracheae, visible both in calcium recordings and the subsequent anatomical stainings. Scale bar = 100 µm. M, medial; D, dorsal.

Figure 5 **(Bottom)** Pairwise comparisons (Spearman rank) of responses to odours and concentrations put into a matrix. Each square represents the average correlation index of eight animals. Correlation indices were colour coded according to the colour scale. Pattern similarity was generally highest between responses to the same compound at different concentrations. In a few instances, correlation was higher across compounds than across concentrations, e.g. between linalool and geraniol at the highest concentration. Increased difference in concentration between the stimuli resulted in weaker correlation.





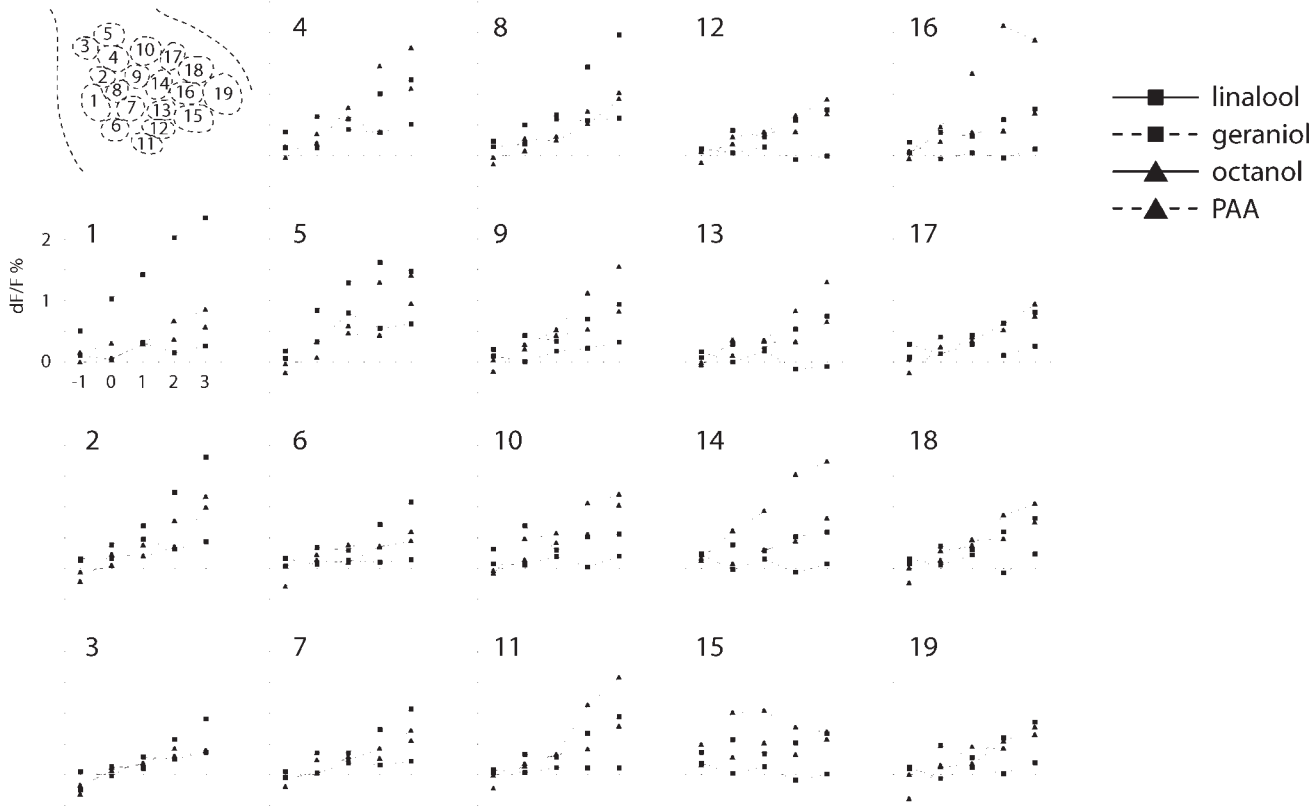


Figure 2 Dose–response curves for linalool, geraniol, octanol and phenylacetaldehyde (PAA) in 19 glomeruli in a single individual (same as in Figure 1). Responses are expressed as percentage change in fluorescence (d*F*/*F*) and shown as a function of concentration (log µg). Note that dose–response functions differ both between compounds and between glomeruli.

Table 1 Movement of the principal glomerulus (the most strongly activated) for each compound across three decadic steps of concentrations (1 µg–1 mg)

Odour	Moving of principal glomerulus across concentrations		To direct neighbour		To proximal glomerulus in the same region		To distant glomerulus	
	%	*n*	%	*n*	%	*n*	%	*n*
Geraniol	50	4/8	25	2/8	25	2/8	0	0/8
Linalool	62.5	5/8	25	2/8	25	2/8	12.5	1/8
Octanol	87.5	7/8	25	2/8	50	4/8	12.5	1/8
PAA	100	8/8	62.5	5/8	37.5	3/8	0	0/8

For each odour the percentages of all animals (eight) demonstrating a movement is shown. A proximal glomerulus is for the alcohols a glomerulus in the lateral part of the lobe close to the antennal nerve, whereas for PAA it is a glomerulus in the medial region of the lobe.

doses used in our experiment. Activity in a few glomeruli increased abruptly, whereafter activity remained at a moderate level with increasing concentration (e.g. glomerulus 15; Figure 2).

Number of activated glomeruli

We counted the number of activated glomeruli and expressed them as the percentage of all visible glomeruli (as observed in subsequent anatomical stainings). A response in a glomerulus was defined as activity exceeding a threshold, set to twice the mean standard deviation of prestimulus activity. The mean percentages of activated glomeruli at five different concentrations are shown in Figure 4. We found that the number of activated glomeruli significantly increased with the concentration [one-way analysis of variance (ANOVA) followed by Tukey–Kramer HSD].

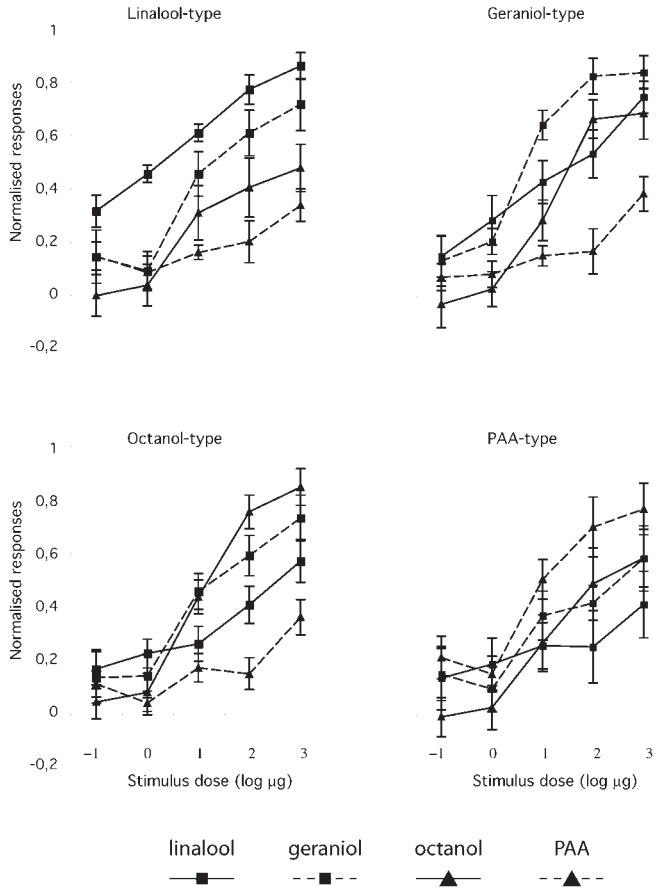


Figure 3 Dose—response curves in four identified glomeruli showing the mean across eight individuals. The glomeruli were identified by their position and responses at 100 µg. In each glomerulus, responses to the four compounds are shown. Bars represent SEM. Responses are normalized by setting the maximal response in each animal to 1. The four identified glomeruli correspond to glomeruli 1 (linalool), 8 (geraniol), 4 (octanol) and 16 (PAA) in the animal in Figure 2.

Percentage data were transformed prior to analysis using the arcsine method (Sokal and Rohlf, 2000). At the lowest dose, activity was observed in <40% of all visible glomeruli. The highest dose used in the experiment resulted in activity in 70–90% of the glomeruli.

Correlation of responses

First, the net change of fluorescence was calculated for all glomeruli. The activity in each glomerulus represented one dimension in a multidimensional space that constituted the response to a stimulus. The responses were normalized (the strongest activated glomerulus for each stimulation was given a value of 1) and compared to all others using a Spearman rank test (JMP, SAS). A linear correlation test (Pearson) provided similar results.

Correlation indices for all pairs of odours and concentrations were calculated and averaged across animals. The values were colour coded and put into a matrix (Figure 5). Similarity was highest between responses evoked by the same compound. The correlation, however, decreased when the difference in concentration between the stimuli increased. The weakest correlations were found between responses to PAA and the alcohols. In a few instances, the glomerular patterns were actually more similar across odorants than across concentrations. For example, the mean correlation index between responses to 1 mg of geraniol and 1 mg of linalool is significantly higher than the index between responses to 1 mg and 1 µg of linalool (0.78 versus 0.42, $P < 0.001$; paired t-test, two-tailed distribution; $n = 8$).Finally, we compared the correlation indices between pairs of odorants at a low (1 µg) and a high (1 mg) dose. A significant increase in correlation at the high concentration for the pairs geraniol–linalool ($P < 0.001$), geraniol–octanol $P < 0.01$) and octanol–linalool ($P < 0.05$) was found (Figure 6; paired t-test, two tailed distribution; $n = 8$). Thus, patterns evoked by these compounds tend to be more similar to each other at high concentrations. In contrast, comparison with patterns evoked by PAA did not render an increased similarity at high concentrations.

Discussion

Odour-evoked calcium signals in *S. littoralis* show a dynamic range of 3–4 log units for the most strongly responding glomeruli. Whereas other studies have reported a concentration-dependent expansion of the focal area of activity due to a recruitment of glomeruli (Rubin and Katz, 1999; Johnson and Leon, 2000; Fuss and Korsching, 2001; Meister and Bonhoeffer, 2001; Wachowiak and Cohen, 2001; Fried *et al.*, 2002), we show that intensity does not simply increase evenly in all glomeruli. Instead, the focus of activity often moves between glomeruli across concentrations. Relative response patterns vary with concentration and get become identical as the difference in concentration increases. Finally, correlation of response patterns evoked by different compounds increases with concentration.

In an earlier study (Carlsson *et al.*, 2002), we could not prove that the observed localized foci of odour-evoked activity originated in single glomeruli. Adopting the post-staining protocol elaborated for honeybees (Galizia *et al.*, 1999) to *S. littoralis*, we can here demonstrate that activity is indeed confined within boundaries of glomeruli.

Dynamic range

The dynamic range of activity in the glomeruli spanned 3–4 log units. A similar range is seen in vertebrate imaging studies (Fuss and Korsching, 2001; Wachowiak *et al.*, 2002). Extracellular recordings from single ORNs in *S. littoralis* have also revealed dose–response dynamics within the same range (Ljungberg *et al.*, 1993; Anderson *et al.*, 1995). In contrast, receptor neurons in vertebrates generally show a much narrower range (Duchamp-Viret *et al.*, 1999, 2000). The expansion of the dynamic range from single ORNs in the periphery to populations of neurons in the vertebrate

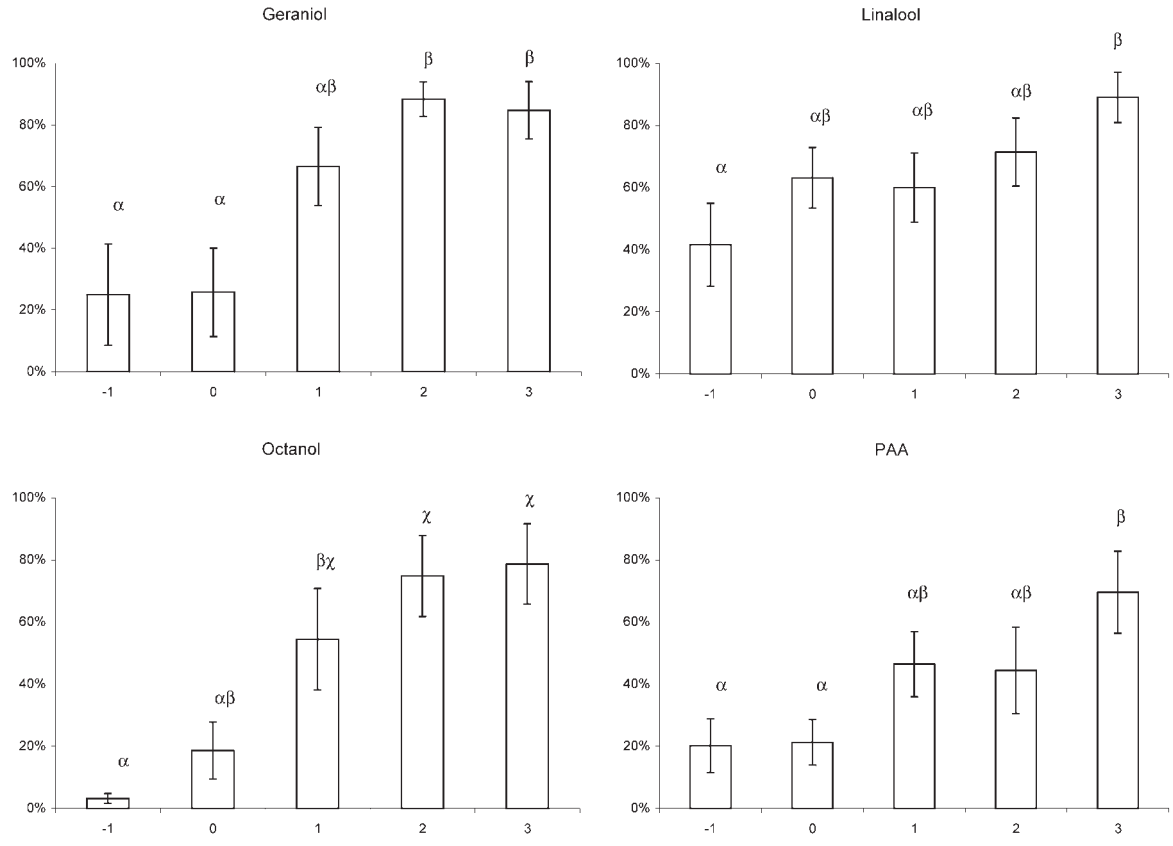


Figure 4 The columns show the percentages of all visible (in anatomical stainings) glomeruli activated by geraniol, linalool, octanol and PAA, respectively, at five concentrations (mean of eight animals ± SEM). The threshold for activation was set to two standard deviations above the background activity (i.e. pre-stimulus activity). Note that this threshold is lower than in Figure 1 and the number of activated glomeruli is thus higher. All odorants showed a significant increase in the number of activated glomeruli (one-way ANOVA, followed by Tukey–Kramer HSD). Columns capped by different letters differ significantly.

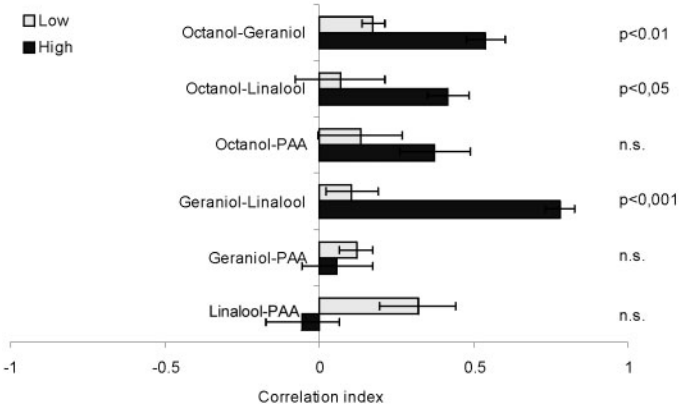


Figure 6 Comparison of correlation indices of glomerular activity patterns evoked by pairs of odorants at a low (1 μg) and a high (1 mg) concentration. Each column shows the mean correlation index (±SEM) of eight animals. For the pairs octanol–geraniol, octanol–linalool and geraniol–linalool, correlation indices were significantly higher at the high concentration (paired *t*-test); n.s., non-significant.

olfactory bulb could be explained if different receptor neurons detecting the same molecules have different threshold and saturation levels and converge on the same glomerulus. This is apparently not the case in insects. The very large family of receptor-coding genes in vertebrates could possibly contain receptors with similar qualitative tuning, but differing dynamic ranges.

Dose–response curves often reached a plateau in the most strongly responding glomeruli, suggesting a sigmoid function. This means that glomerular responses (and likely ORNs) saturate at high concentrations. Sigmoid dose–response functions are often observed in single antennal neurons (Ljungberg *et al.*, 1993; Anderson *et al.*, 1995). Some glomeruli, however, showed moderate activity at low concentrations, but no further increase at higher doses (see below).

Activity focus

We showed in an earlier study (Carlsson *et al.*, 2002) that even if odour-evoked activity was distributed in a large number of glomeruli, each odorant preferentially activated a single glomerulus (or a few glomeruli). A single concentration was, however, used for all compounds tested. Here, we show that the principal glomeruli are often not

consistent across concentrations, but rather move to neighbouring glomeruli. A focal movement is the result of different concentration-dependency among glomeruli. Saturation is reached at different concentrations and the slopes of the dose–response curves differ. A possible explanation of why a focal shift of activity generally resulted in a neighbouring glomerulus becoming the most activated is that ORNs expressing receptor molecules with overlapping receptive ranges project to adjacent glomeruli. In fact, axons of vertebrate ORNs expressing homologous and closely linked receptor genes arborize in neighbouring glomeruli (Tsuboi *et al.*, 1999). Concentration-dependent movement of principal glomeruli has also been observed in mice (Fried *et al.*, 2002). In the latter study, ORNs were selectively stained with a calcium-sensitive dye. This means that the focal shift in the mouse was exclusively attributed to activity in the ORN terminals. In honeybees, on the other hand, the same principal glomeruli persisted across concentrations (Sachse *et al.*, 1999). However, we used a wider range of concentrations and far lower doses and the results can thus not be directly compared.

Glomerular recruitment

With higher stimulus concentration, an increasing number of glomeruli were activated. It is remarkable that even at the lowest concentration, linalool, for example, excited ~40% of all visible glomeruli and at the highest concentration virtually all. This could, to some extent, be due to responses in multiglomerular local interneurons (LNs) or responses in projection neurons (PNs) arborizing in glomeruli not innervated by responding ORNs. Even though a bath application of the calcium-sensitive dye is non-selective with respect to the type of neuron, afferent-selective staining methods have also revealed responses in a very large population of glomeruli (Friedrich and Korsching, 1997; Fuss and Korsching, 2001; Wachowiak and Cohen, 2001; Fried *et al.*, 2002) The increase in the number of responding glomeruli with concentration likely reflects a recruitment of ORN types detecting a specific compound. These ORNs have a low affinity and are only activated at high concentrations. Plant-odour-responding ORNs in *S. littoralis* respond to a broader spectrum of compounds at higher concentrations (Anderson *et al.*, 1995). Even pheromone-specific neurons can respond to non-pheromones if the concentration is high enough (Hansson *et al.*, 1989; Carlsson and Hansson, 2002). A recruitment of responding glomeruli with increasing concentration has also been observed in optical imaging studies in vertebrates (Rubin and Katz, 1999; Johnson and Leon, 2000; Fuss and Korsching, 2001; Meister and Bonhoeffer, 2001; Wachowiak and Cohen, 2001; Fried *et al.*, 2002) and, recently, in fruitflies (Wang *et al.*, 2003).

Correlation of glomerular patterns

When odour-evoked activity patterns for different concentrations of the same compound were normalized (i.e. not considering absolute intensity), it became clear that the relative patterns were not identical. Instead, we found a decrease in pattern similarity when the difference in concentration between the stimuli increased. Furthermore, as has been reported in vertebrates (Fuss and Korsching, 2001), we found that activity patterns evoked by different compounds become more similar at high concentrations. However, this effect seems to depend on the similarity of the odorants, i.e. if they overlap in molecular structure. Geraniol, linalool and octanol all have an attached alcohol group as a common denominator and do not have a cyclic structure, whereas PAA consists of a benzene ring with an attached aldehyde group.

Recently, it was found (Ng *et al.* 2002), using a binary glomerular coding scheme, that the distance between responses to different compounds actually increased with concentration in *Drosophila*. The stimuli used were fruit odours that are complex mixtures of volatiles. Thus, differing analysis methods and choice of stimuli make it difficult directly to compare the results.

Several different factors may contribute to the concentration-dependent pattern changes. First of all there is a recruitment of glomeruli at increased concentration (as discussed above). Secondly, activity in insect ORNs saturates at high stimulus concentrations due to adaptation (Todd and Baker, 1999). If an ORN is saturated by its key compound, other neurons may respond equally strongly and the original across-neuron ratio changes. Furthermore, when an ORN is saturated at high doses by its key compound, a second-best stimulus may excite the neuron equally well. Geraniol, linalool and octanol activate overlapping subsets of ORNs in *S. littoralis* (Anderson *et al.*, 1995). This means that the ratio between the active neurons may change at high concentrations and the odour-unique, across-neuron pattern may become less distinctive for these compounds. The levelling concentration–response functions (Figure 2) show that activity in certain glomeruli saturates at high concentrations. Thirdly, in some glomeruli saturation was already reached at intermediate doses. One reason for this may be that responding ORNs do not directly innervate these glomeruli. Instead, the signals observed might come from LNs, connecting glomeruli innervated by responding ORNs and those that are not. LNs connect either most or all glomeruli in the lobe with equally dense innervation, or connect only a few glomeruli with innervation biased to a single glomerulus (Anton and Homberg, 1999). Even though calcium imaging, using a bath application of the dye, emphasizes ORN activity (Galizia *et al.*, 1998), part of the measured signal likely derives from AL interneurons. In contrast to ORNs, LNs and PNs are often much less concentration-dependent and many interneurons do not express increased spike activity at concentrations above threshold levels (Anton *et al.*, 1997; Masante-Roca *et al.*, 2002). An alternative explanation for saturation at inter-

mediate concentrations is that responding ORNs innervate these glomeruli, but are presynaptically inhibited, a mechanism demonstrated in crustaceans (Wachowiak and Cohen, 1998, 1999).

Olfactory coding and perception

Concentration changes affect not only the intensity of signals in the glomeruli, but also the spatial distribution of activity. Odour-evoked representations in different combinations of glomeruli have been suggested to be a code for odour identity (Johnson *et al.*, 1998; Galizia *et al.*, 1999), which would then be decoded in higher brain centra by computing the ratio of activity among the glomeruli. If such a ratio varies due to fluctuations in concentration, then the decoding of the odour identity may be complicated. Assuming that a combinatorial glomerular code underlies the qualitative perception of an odour, confusion may occur when trying to identify an odour at different concentrations. Perceived odour quality in humans has been shown to vary with concentration (Gross-Isserof and Lancet, 1988; Arctander, 1994). However, it is not a common phenomenon as most odours are described as qualitatively invariant regardless of concentration. Honeybees can easily be trained to respond to odours and by using a differential training paradigm, with one odour coupled with a reward and the other not, discriminatory ability can be studied. Bhagavan and Smith (Bhagavan and Smith, 1997) showed that bees trained to a high concentration of an odorant had a lower tendency to generalize between the trained and a novel odour than bees trained to a low concentration. This indicates that the discriminatory ability actually increases at high concentrations. Even though this seemingly contradicts our results, precise information about the odour quality may still be present in the population of PNs. On the other hand, it cannot be ruled out that the spatial patterns of glomerular activity play a less important role in odour encoding than the temporal characteristics of the signals. If this is so, odour quality could be correctly coded in the temporal patterns at different concentrations, no matter if the spatial patterns vary. Recently, Spors and Grinvald (Spors and Grinvald, 2002) demonstrated in rats, using a fast voltage-sensitive dye, that even though glomerular recruitment occurred, the added glomeruli had longer latencies and the initial pattern and the sequence of glomerular activation were conserved across concentrations. Thus, Spors and Grinvald suggested that qualitative information is contained in the sequencing of activity, whereas information about concentration is carried by the latencies. Analysis of dynamic aspects of the glomerular patterns was, however, limited by the temporal resolution in our experiment.

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