Results

How does the olfactory system encode odor identity across concentrations? Is the similarity between odors altered when the odor concentration changes? We tried to answer these questions using optical recording techniques on our model system, the honeybee AL during stimulation with 16 different odors at 4 concentrations (10^{-4} to 10^{-1}, diluted in mineral oil). Therefore, we selectively stained uniglomerular l-ACT PNs of the AL with the calcium reporter Fura2 dextran and measured the changes in fluorescence. Changes in intracellular calcium reflect changes in PN activity. L-ACT PNs innervate glomeruli which lie on the dorsal side of the AL and are named according to the tract which sub serves them as T1-1 to T1-64 (Galizia *et al.*, 1999a). Throughout the rest of this manuscript, we will leave out the T1 and will refer to them only by their number.

The odors used were hydrocarbons belonging to four different functional groups (primary and secondary alcohols, aldehydes and ketones) of 4 different carbon chain lengths (C6 to C9). Raw recordings were transformed into correlation images (see Material&Methods) in which glomeruli were nicely visible, and could be identified according to their morphological borderlines using the digital atlas of the AL as a reference (Galizia *et al.*, 1999a). Between 10 and 22 glomeruli (mean=17.6+-2.1) could be reliably identified in each animal. Subsequently we will limit our analysis to the 14 most commonly identified glomeruli.

PN activity patterns exhibit complex temporal dynamics

Upon stimulation with an odor, characteristic spatiotemporal changes in fluorescence intensity were observed. These changes were truly odor responses, as they were not visible when stimulating with mineral oil or air alone. 88% of all responsive glomeruli showed an increase in activity while the rest showed a decrease. While most glomeruli responded to odors with an increase in activity, some could also respond with a decrease, depending on the odor. However we observed no glomeruli which responded with a decrease in activity to all tested odors. The response strength varied between

animals, glomeruli and odors. The maximal and minimal changes in fluorescence measured were 24.4% and -14.4%, but generally changes were smaller.

Glomerular responses were also temporally dynamic. The response onset could differ for different glomerulus/odor combinations. While many glomeruli would react instantly to odors, some entered the pattern after a delay of up to 400 ms. Additional measurements performed at a higher frequency (20Hz) confirmed that the response onset did not solely depend on the response strength, as some strongly responding glomeruli entered the activity patterns consistently later than others (data not shown).

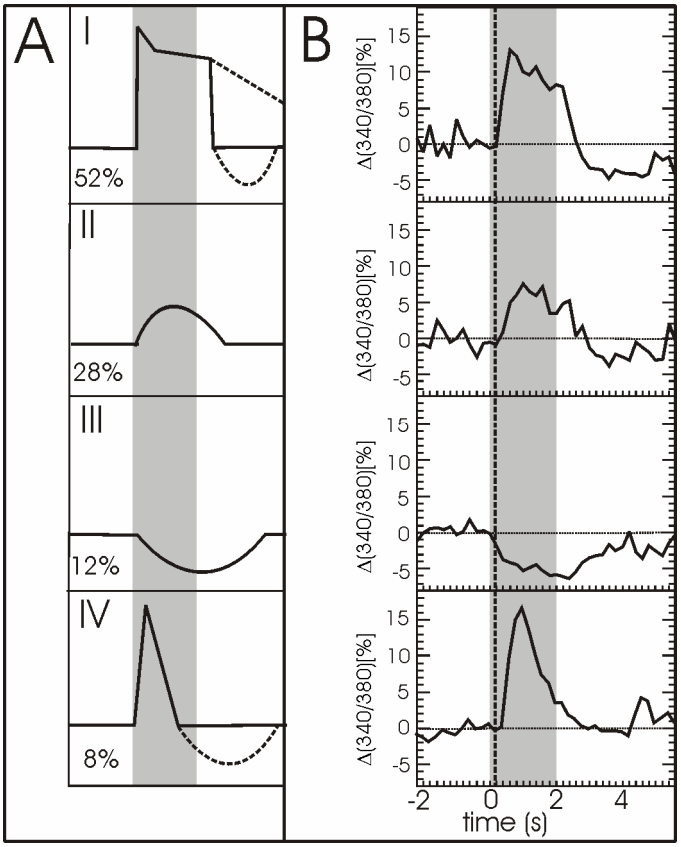

Figure 1) PN responses could be divided into 4 different types. PN responses are plotted over time. Grey bar represents the stimulus. A) Schematic drawings of the 4 general response types together with their relative frequency. Dotted lines indicate alternative courses after stimulus offset. B) Exemplary measurements obtained from glomerulus 29 in different odors at 10^{-1}. From top to bottom: 2-octanone, 2-octanol, 1-octanol and 2-nonanone. The dashed line shows the response onset of the type I response.

According to their shape, but independent of their onset, responses could be classified into four general types (Fig. 1):

I) 52% of all glomerular responses showed a “phasic-tonic” increase in intracellular calcium. These responses were strong, often surpassing 10% change in fluorescence. In some cases the tonic component increased with odor concentration. The response offset was variable: While in most cases calcium concentration went back to baseline after stimulus offset, some even showed an post stimulus undershoot. In other cases the response hardly followed the stimulus offset the calcium level remaining high throughout the end of the measurement.

II) 28% of all glomerular PN responses were weak (below 5 %), especially when odors were presented at lower concentrations. These responses did not have a clear cut shape. Glomeruli which showed type II responses at low odor concentrations, often showed type I responses when activated by the same odor at higher concentrations.

III) 12 % were inhibitory responses. They typically showed a gradual decrease in calcium concentration starting with stimulus onset. At stimulus offset the calcium concentration was at its minimum, increasing in the absence of the odor as gradually as it had decreased before. The absolute fluorescent change was generally small, only in some cases up to one third of the strength of the “phasic-tonic” responses described above.

IV) 8% of all glomerular responses were phasic. They showed a strong excitation, quickly followed by a strong decrease in calcium. In some cases the decrease in calcium could pass the baseline resulting in a post stimulus inhibition.

The distribution of the different response types over the concentrations is shown in table 1.

The same glomerulus showed different response types, depending on the odor presented (Figure 1B). In some cases, we observed off responses as described previously (Sachse and Galizia, 2002). These responses were rare (less than 1% of all odor responses) and could only be seen at the highest odor concentrations.

Table 1) Number of different response types for the four concentrations measured. Response type classification was done on odor responses averaged across animals. Rows show response types, columns concentrations.

	10^{-4}	10^{-3}	10^{-2}	10^{-1}	Σ
I	17	38	61	81	197
II	19	26	27	34	106
III	4	5	14	25	48
IV	2	1	13	14	30
no response	182	154	109	70	515

PN activity patterns are variable

As found in previous studies (Ng *et al.*, 2002; Sachse and Galizia, 2002; Wang *et al.*, 2003), PN activity patterns were conserved across animals. Figure 2 shows the responses to 2-heptanone in three different animals. Glomeruli dominantly active upon stimulation with an odor in one animal were active in other animals, too. Nevertheless, both the absolute and the relative strength of glomerular activation varied between animals. For example in the first animal of Figure 2 glomerulus 60 (brown line) was as strongly activated as glomerulus 36 (pink line), while it was weaker in the other two animals. In some extreme cases, glomeruli would even be absent in the patterns. Glomerulus 38 (yellow line),a glomerulus which also responds to 1-hexanol, for example was strongly active in eight out of nine animals tested with 2-heptanone. In one case though, it was not active at all (animal 3 in figure 2). Since in this animal the glomerulus responded also to 1-hexanol (data not shown), we can exclude damage to the glomerulus or wrong identification.

As mentioned this example was a rather rare case. When later averaging the glomerular responses across animals, the resulting standard errors were rather small (Figure 4).

Chemical odor similarity is contained in the glomerular PN activity patterns

Glomerular activity patterns evoked by chemically similar odors, like 1-hexanol and 1-heptanol seemed to have a greater overlap than more distinct odors. To further investigate and quantify this similarity, we used a linear discriminant analysis (LDA).

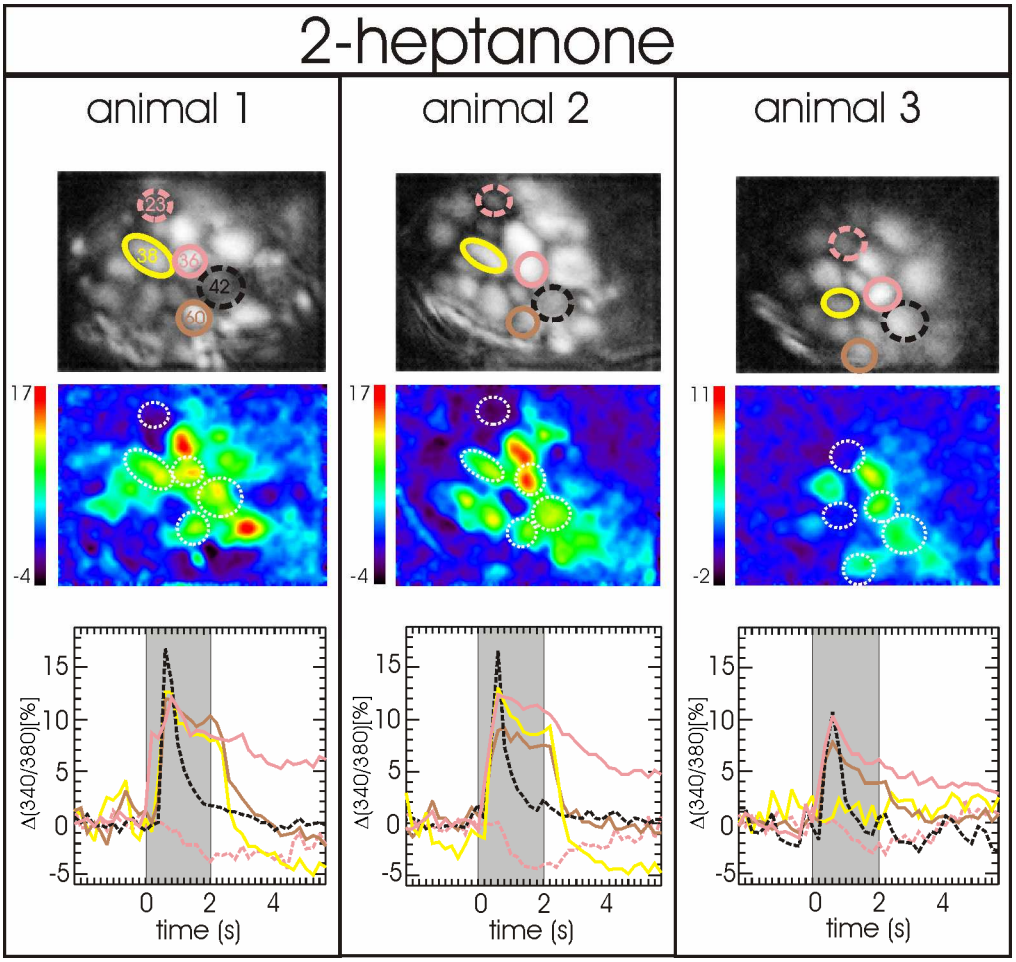

Figure 2) The same odor elicits comparable PN activity patterns in different animals.
Correlation images, glomerular activity patterns and time courses of glomerular responses in three different animals. The presented odor was 2-heptanone at a concentration of 10^{-1}. PN responses in different animals show variations in their relative and overall strength. Glomerulus 38 in animal 3 is an example for a glomerulus which is generally activated by this odor, but is missing in this pattern.

LDA is a statistical method for supervised classification which functions by increasing the ratio of between group variance to within group variance. We translated each of our 472 single odor measurements into a vector with 14 elements, where each element corresponds to the averaged activity of one glomerulus during stimulus. In the classification procedure these vectors had to be assigned to one out of 64 groups, corresponding to the 16 odors x 4 concentrations. The LDA tries to find transformations based on common characteristics in the vectors belonging to the same group, and on the differences between those which do not belong together. As a rule of thumb, LDA

performs well when the number of different groups (in our case 64) is much smaller than the number of vector elements (the 14 glomeruli). Since this was not the case, we did not expect the LDA to perform well. However we wanted to know to what extent such a classification was possible and in which cases the classifications failed. These results can be taken as a direct measure of the similarity between glomerular activity patterns.

53% of all measurements were correctly classified according to their odor and concentration. In 70%, the LDA correctly classified the measurements according to the odor, but not to the correct concentration. 88% of all measurements were classified to odors having the same chain length and in 98% to odors having the same or a neighbouring chain length. In 86% of all measurements, the LDA classified the measurements to the correct functional group. We conclude that chemical similarity/difference between odors is maintained in the PN activity patterns.

Intracellular PN calcium changes with concentration

The number of active glomeruli increased with concentration. At the lowest concentration (10^{-4}), a mean of 19% of the identified glomeruli were activated. Increasing the concentration, this number increase to a mean of 31% (10^{-3}), 51% (10^{-2}) and 68% (10^{-1}) (See table 1). Generally, all PNs activated by an odor at a low concentration were also activated by the same odor at higher concentrations. This was true both for changes caused by an increase and those caused by a decrease in calcium. Additionally, the strength of glomerular activity increased steadily. Figure 3) shows the responses of all identified glomeruli to 2-octanone at the four different concentrations. At the lowest concentration, only three glomeruli, 17 (black line), 33 (blue line) and 36 (pink line) were active. The responses were weak; hardly reaching 5% and could be classified as type II. Increasing the concentration to 10^{-3} strongly increased the activity in glomerulus 36, which now showed a type I response. Additionally, glomerulus 52 (cyan line) became activated. At a concentration of 10^{-2}, glomerulus 28 (red line) became strongly activated, while glomerulus 36 increased its activity only slightly. Also glomerulus 29 (green line) entered the pattern, exhibiting a type IV phasic response. Glomerulus 48 (green dashed ine) in turn showed an off response. At the highest concentration (10^{-1}), many additional

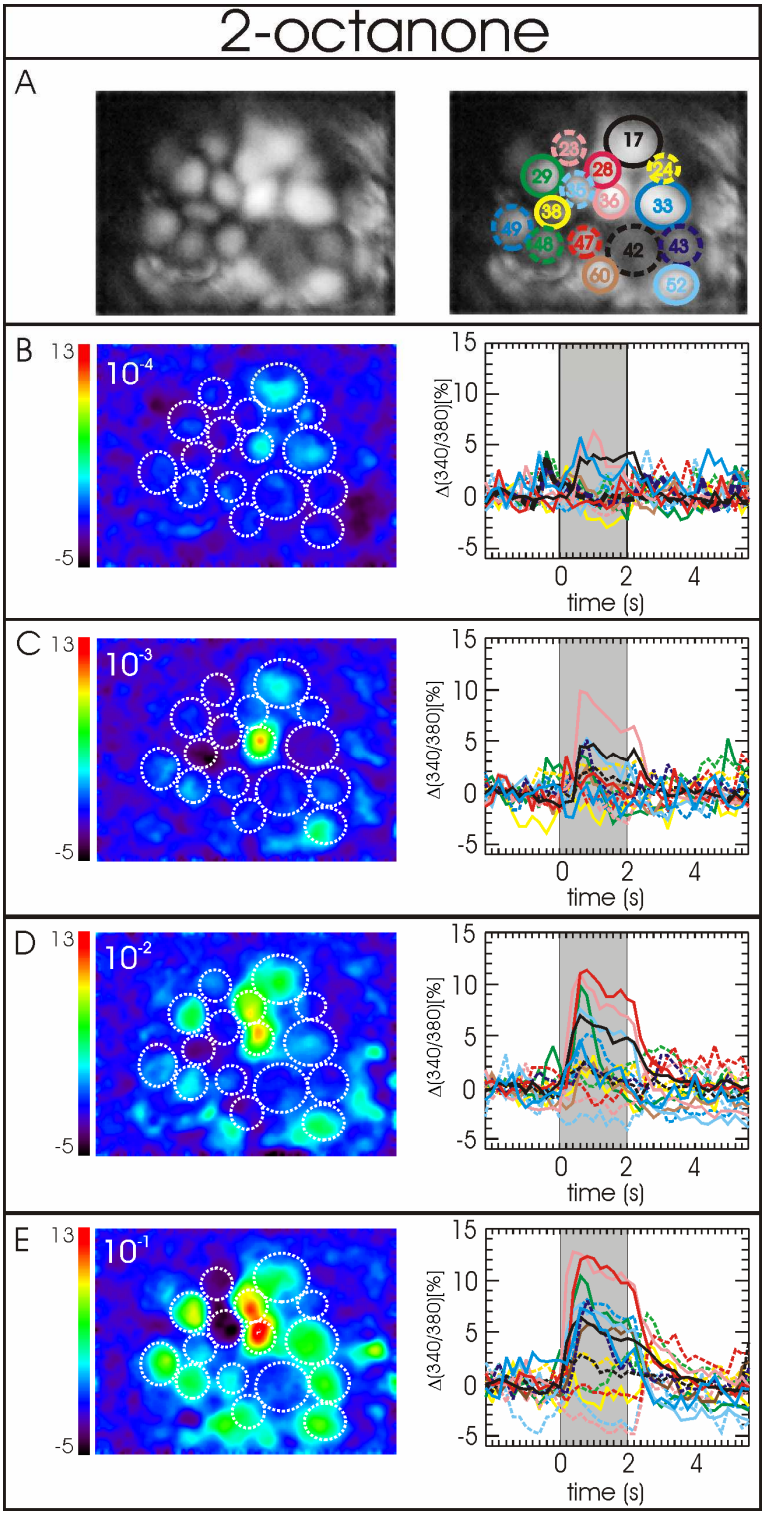

Figure 3) Effect of odor concentration on PN activity. A) Correlation image of the AL and the same image with overlaid glomerular identity. B-E) Glomerular activity patterns and glomerular responses plotted over time as a response to 2-ocatnone presented at increasing concentrations. PN activity elicited by higher odor concentrations are a superset of the patterns elicited by the same odor at lower concentrations. PN activity increases over concentrations.

glomeruli were active. While glomerulus 29 now showed a type I response, the off response of glomerulus 48 increased in strength. In some cases glomeruli did not linearly increase their activity with increasing odor concentrations: Glomerulus 33 (blue line) for

example responded very weakly when stimulated with 2-octanone at a concentration of 10^{-4}, this response disappeared when increasing the concentration to 10^{-3} and reappeared at higher concentrations.

Since we assume that the glomerular PN patterns are conserved across animals, we subsequently averaged our data across animals. Five glomeruli, namely 17, 28, 33, 36 and 52, dominated the PN calcium patterns in response to all odors. These glomeruli were the only ones active when odors were presented at the lowest concentration. When increasing the concentration to 10^{-3}, 88% of all active glomeruli did belong to this set of five. This indicates that these glomeruli are specifically sensitive to the tested set of odors. Further increments in concentration evoked responses also in the other nine glomeruli. Figure 4 shows the mean activity of these five glomeruli to all odors across concentrations, averaged over the animals. Generally, the five most sensitive glomeruli mentioned above were more strongly activated than most other glomeruli, and their responses covered a large part of their dynamic range.

However, in some cases they were already very strong when stimulated with odors at the lowest concentration (see glomerulus 28 in 2-hexanol) and did not increase any further when increasing the odor concentration. The relative glomerular activity changed over concentrations and in several cases glomeruli which had been weaker than others at lower concentrations, became stronger at higher concentrations. The peculiar decrease of the response of glomerulus 33 (blue band) to 2-octanone at 10^{-3} could be seen here, too, this time averaged across ten animals. Although weak, such an effect was visible in several other cases, too. Another finding was the relative decrease in activity of glomerulus 17 to 2-nonanol at 10^{-1} as compared to 10^{-2}.

Figure 5 shows the glomerular PN responses over time, averaged across animals. In most cases glomerular activity patterns evoked by an odor at a low concentration were a subset of the activity patterns evoked by the same odor at higher concentrations. The responses in many glomeruli became longer with increasing concentrations. At the same

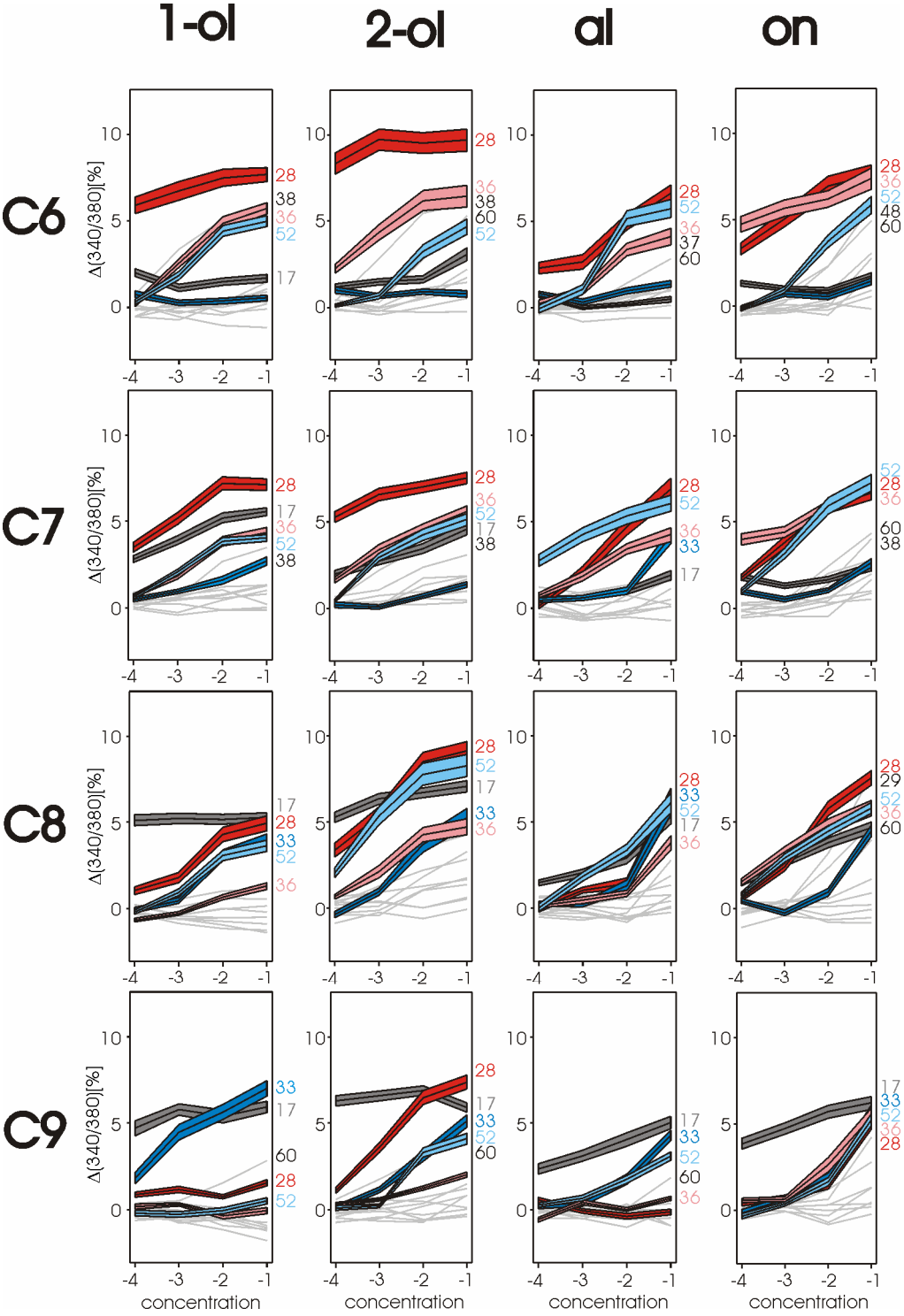

Figure 4) Dose response curves of 14 identified glomeruli to all odors presented. Each subplot represents one odor. Row names depict carbon chain length, column names the functional groups (1-ol =primary alcohol, 2-ol=secondary alcohol, al= aldehyde, on =secondary ketone). Shown are the mean responses during stimulus, averaged across animals. Standard errors of the 5 most sensitive glomeruli are shown as colored bands: gray=17, red=28, dark blue=33, pink=36 and cyan=52. Mean values of the other glomeruli

are shown in grey. The names of the five glomeruli showing the strongest responses are shown to the right (ordered from bottom to top with increasing activation strength).

time, more glomeruli responded with a decrease in intracellular calcium both during and after the stimulus. In three cases (glomerulus 42 in hexanal, glomerulus 48 in octanal and glomerulus 49 in 2-octanone), off responses could be observed. These off responses appeared only at the highest odor concentration.

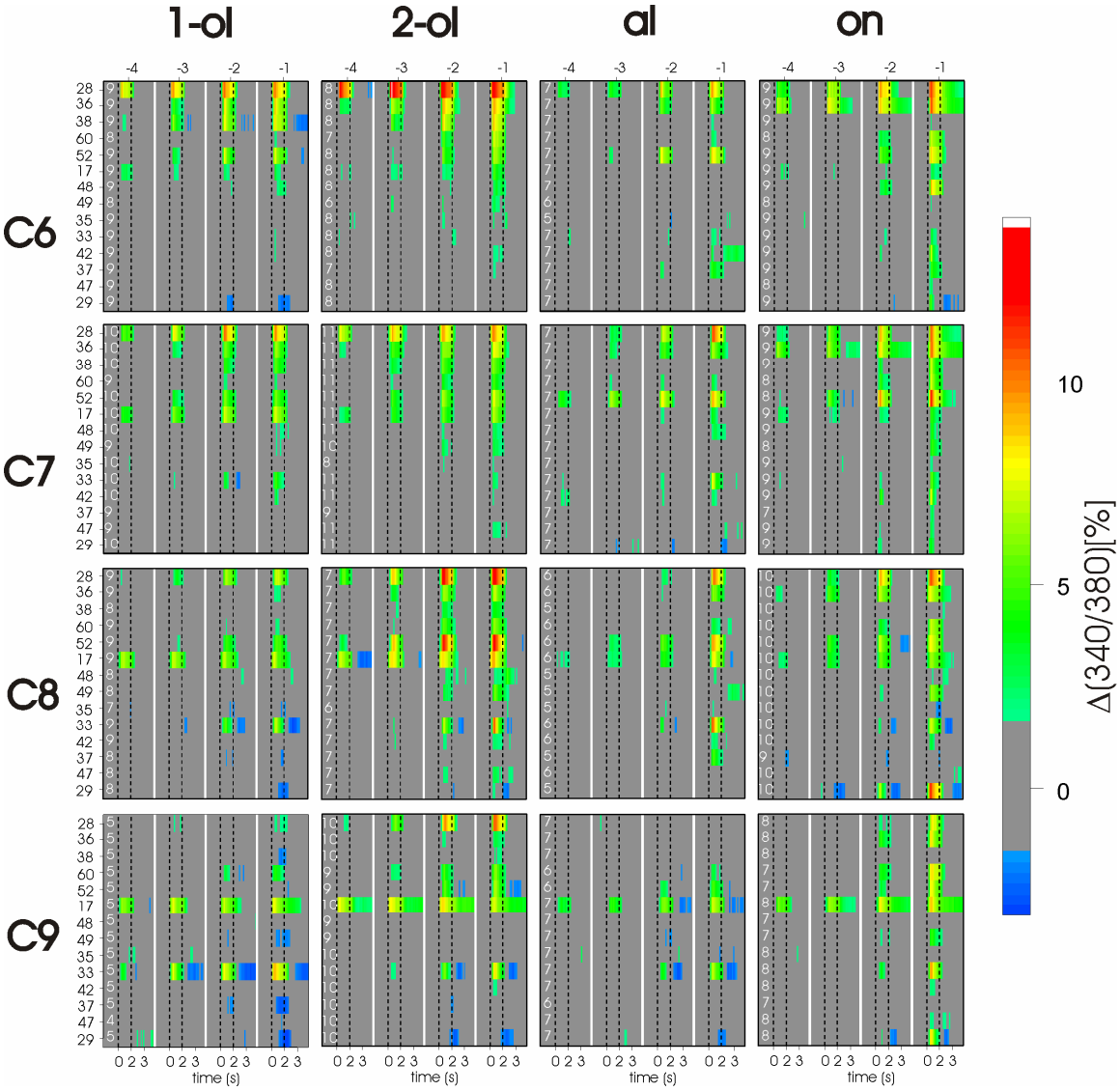

Figure 5) Average PN activity over time. Subplots are arranged as in Figure 4). Each subplot consists of 4 vertical bands, separated by white lines. Bands represent the activity of 14 identified glomeruli over time for each of the 4 concentrations tested. Striped lines within each band delimit the stimulus duration.

Absolute values smaller than a threshold of 1.75% Δ(340/380)[%] are gray shaded. White numbers depict the number of animals this glomerulus was identified in. The glomeruli are sorted according to their strength of activation by 2-hexanol.

Glomerular PN activity patterns are odor and concentration specific

In general, each odor differentially activated several glomeruli, resulting in unique, odor specific patterns. Only for some odors at low concentration (2-nonanone, nonanal and octanal, all 10^{-4}) glomerulus 17 alone was active, though to different degrees (figure 6). Stimulation with 2-nonanone resulted in a maximal calcium signal of 6%. The two aldehydes elicited smaller signals (2% for octanal and 4% or nonanal). When increasing the concentration of octanal to 10^{-3}, glomerulus 52 joined 17 resulting in a unique pattern. Such unique patterns were achieved for the other two odors only after increasing the concentration to 10^{-2}. Since these exceptions represent a very low percentage of the odors×concentrations tested, and we only measured 14 out of ~160 glomeruli, we cannot conclude that differentiating between these odors represents a challenge for the olfactory system of the honeybee. The order in which the different odors are presented in figure 6 represents the ratio of activity in the glomeruli 28 and 17. We could observe that this ratio decreased with chainlength.

Odor identity across concentration can only be extracted using multiple linear functions

As shown above, the glomerular PN activity patterns were unique for most odors and concentrations. Since a previous study suggested that odor quality is encoded in a concentration invariant way by the relative glomerular activity (Sachse and Galizia, 2003), we studied this aspect in more detail. As observed in figure 4 there was no general rule according to which the relative glomerular PN activity evolved over concentrations, thereby indicating odor identity. For many odors, the relative glomerular activity changed with concentration. Also when ranking the glomeruli according to the strength of their activation, no concentration invariant scheme became visible (data not shown).

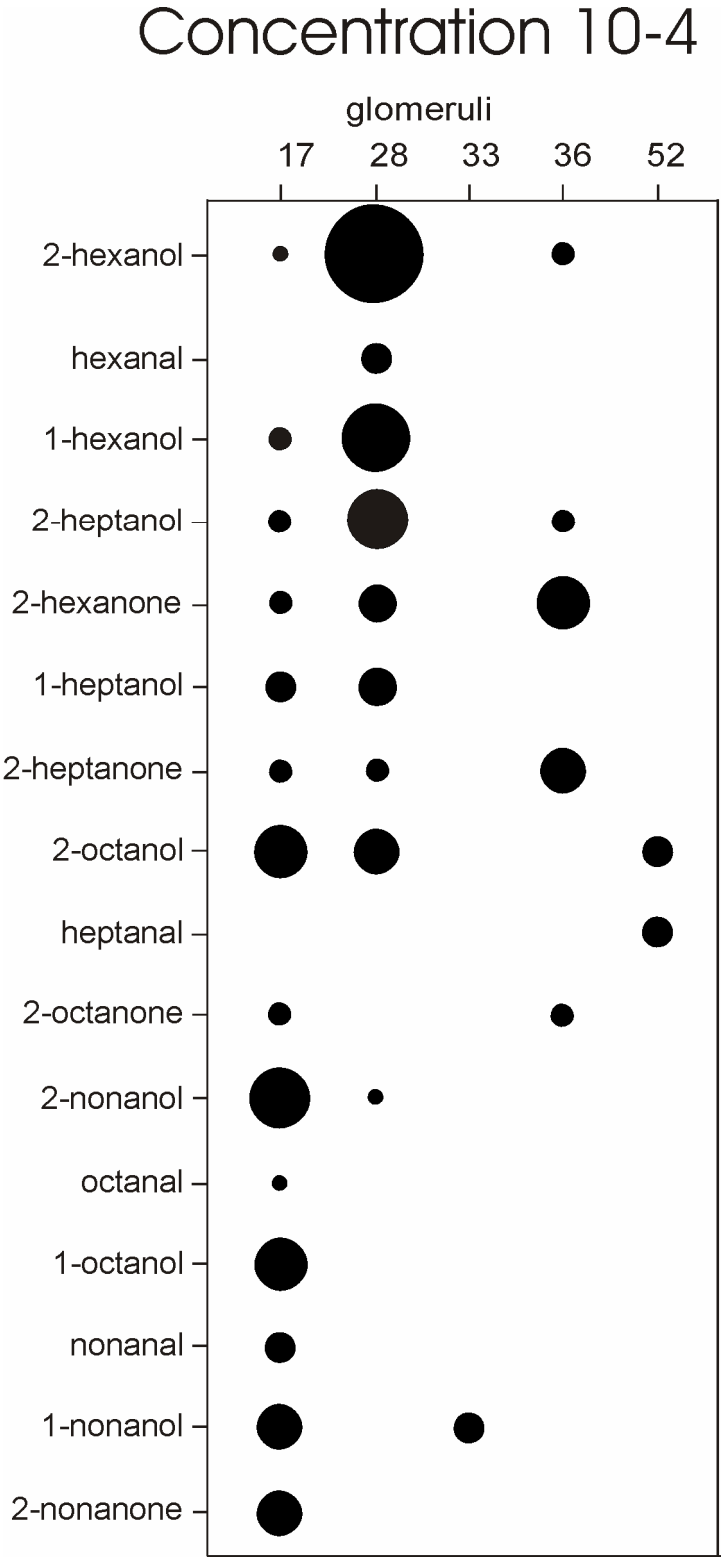

Figure 6) Responses of the 5 most sensitive glomeruli to the lowest odor concentration (10^{-4}), averaged over the stimulus duration. Columns represent the glomeruli, rows the odors. Responses below 1.75% Δ(340/380)[%] are not shown. Circle size reflects the strength of activation. Odors are ordered according to the ratio between glomeruli 28 and 17.

Next we tested, whether a concentration invariant classification based on the glomerular activity patterns was possible at all. We resorted to the LDA, this time in

order to classify the odors irrespective of their concentration. We used the mean responses averaged across animals as vectors (16x4 vectors) and the 16 odors as groups. And indeed, a concentration invariant classification based on the glomerular activity patterns was possible. Except for one case, in which heptanal 10^{-4} was classified as octanal 10^{-4}, all glomerular patterns were correctly classified. However the transformations required were not simple. Though on all linear discriminants (LDs) the different concentrations of the same odors clustered (Figure 7), a separation of different odors was only possible in a high dimensional space defined by 13 LDs.

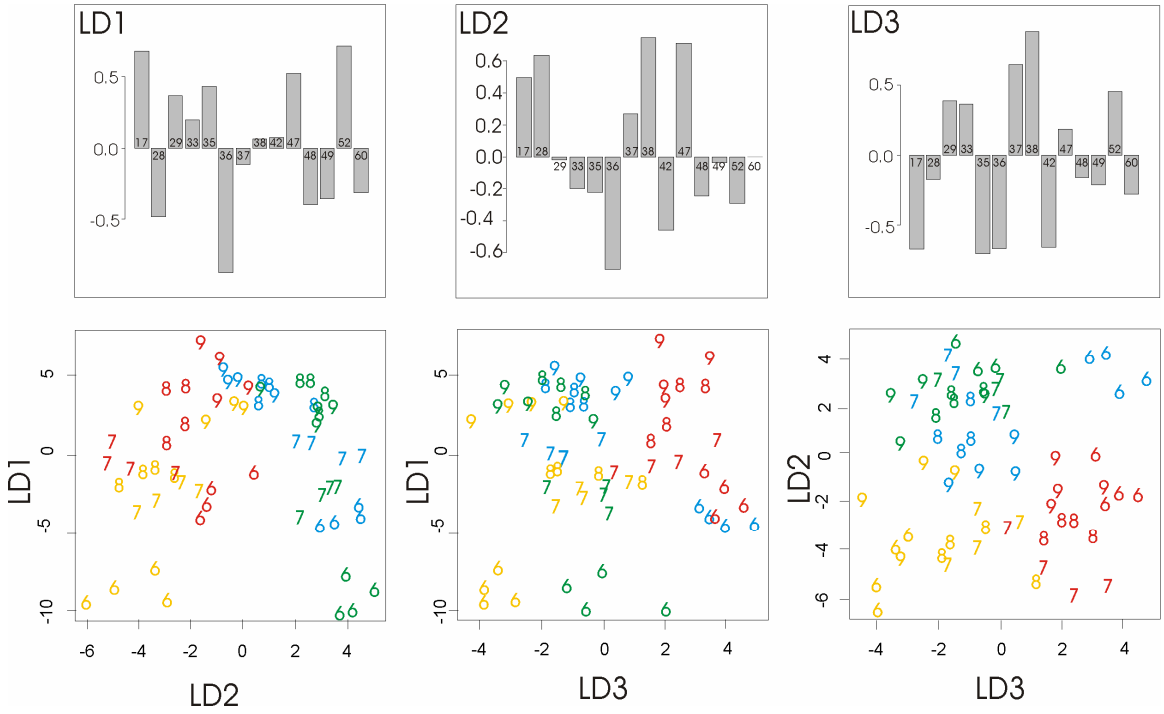

Figure 7) Linear discriminant analysis. Odor representations of all 4 concentrations projected onto three linear discriminants are shown. The bar diagrams show the transformation to the linear discriminants, which project the data onto the axes as defined by the following formula: $odor_{LDX} = \sum_{glom=1}^{14} (LDX_{glom} \times odor_{glom})$, where $odor_{LDX}$ is the postion of the odor on the xth linear discriminant. *LDX* is the transformation to the discriminat function and *glom* the 14 glomeruli. $odor_{glom}$ is the activity of the specific glomerulus to this odor. Axes of the scatter plots are the first three linear discriminants. Odor chain length is given by the numbers, functional groups are depicted in color: blue=primary alcohol, green=secondary alcohol, red=aldehydes, orange=ketone.

Information about carbon chain length and functional group is encoded in the glomerular patterns

Further exploring the data, we wanted to 1) investigate the trajectories of odor representations over time and 2) explore the relative positions of the odors in the olfactory space. To do so, we used a principle component analysis (PCA). Unlike the LDA, which is used to classify data, this procedure rearranges the data space according to the sources responsible for most variability. In cases where the first three resulting principal components (PCs) are responsible for a high degree of the overall variance, this method allows the visualization of high dimensional data in a 2D or 3D principal component space.

First, we applied the PCA to the odor evoked glomerular responses over time. Figure 8A shows the trajectories of the responses to 1-heptanol and 2-heptanone at different concentrations. Before stimulus onset all odor representations clustered around the origin defined by the PCs. Upon stimulation, the representations of the different odorants projected quickly along PC1 and reached their maximum displacement already 800 ms after onset of the two second stimulus. Then the odor representations started a slow return to the origin, although the stimulus was still present. On their way back, some projections even passed the origin, while others did not reach it before the measurement ended. These differences in the trajectories at odor offset can be attributed to the different response decay times in different glomeruli for different odors. The distance between the origin and the point of maximum trajectory displacement increased with concentration, but all concentrations reached this point at the same time (Figure 8B). The general trajectory directions were dominated by the odor identity, while odor concentration had a minor effect. This resulted in an increasing distance between odor representations, which was maximal when the trajectories reached their maximal displacement (Figure 8C). PC1 obviously correlated with the global glomerular activity and was of comparable strength for all 16 odorants. PC2 and PC3 were mainly responsible for separating the representations according to the odor.

Second, we examined the locations of the individual odors in space (Figure 9). Therefore we repeated the PCA feeding it the mean glomerular odor representations for

each frame recorded separately. Already 600ms after stimulus onset, and throughout the

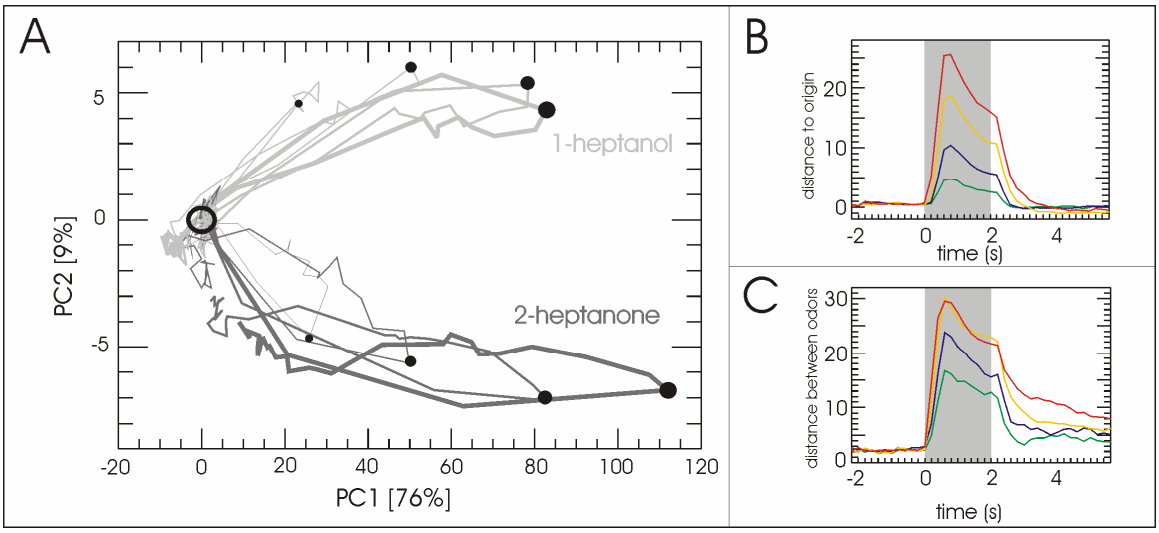

Figure 8) Trajectories of odor responses in the principal component space. A) Exemplary graph of the trajectories of two odors, 1-heptanol and 2-heptanone, over time. Axes are the first two principal components. Line and circle width increase with concentration. Circles show the time point of 800ms after stimulus onset. Empty circle marks the origin. Odor identity has the most dominant influence on the trajectory direction, odor concentration on the trajectory length. B+C) Euclidean distances averaged for all odors. Colors represent the different odor conconcentrations: red=10^{-1}, orange=10^{-2}, blue=10^{-3} and green=10^{-4}. B) Mean Euclidean distance between odor trajectories, separately for each concentration, and the origin, plotted over time. Mean response distance from origin increases during stimulation
C) Mean Euclidean distance between the trajectories of all 16 odors again separated for each concentration, over time.

stimulus, the representations of odors at 10^{-4} and 10^{-3} were separated according to their chemical properties. The first PC separated the different odor representations according to their carbon chain lengths. The second PC separated primary and secondary alcohols from aldehydes and ketones, while the third separated aldehydes from ketones. No such separation could be found for primary and secondary alcohols. The same observation could be made for the two higher odor concentrations, though already 200 ms earlier but became less distinct during the subsequent frames recorded (data not shown).

We visualized the results of our PCA using a Gabriel biplot (Figure 9). This plot has the advantage of combining both the principal component and variable space. Therefore, both the separation of the odors and the different glomerular contributions to

this separation can be visualized. We found that at low concentrations, the distribution of

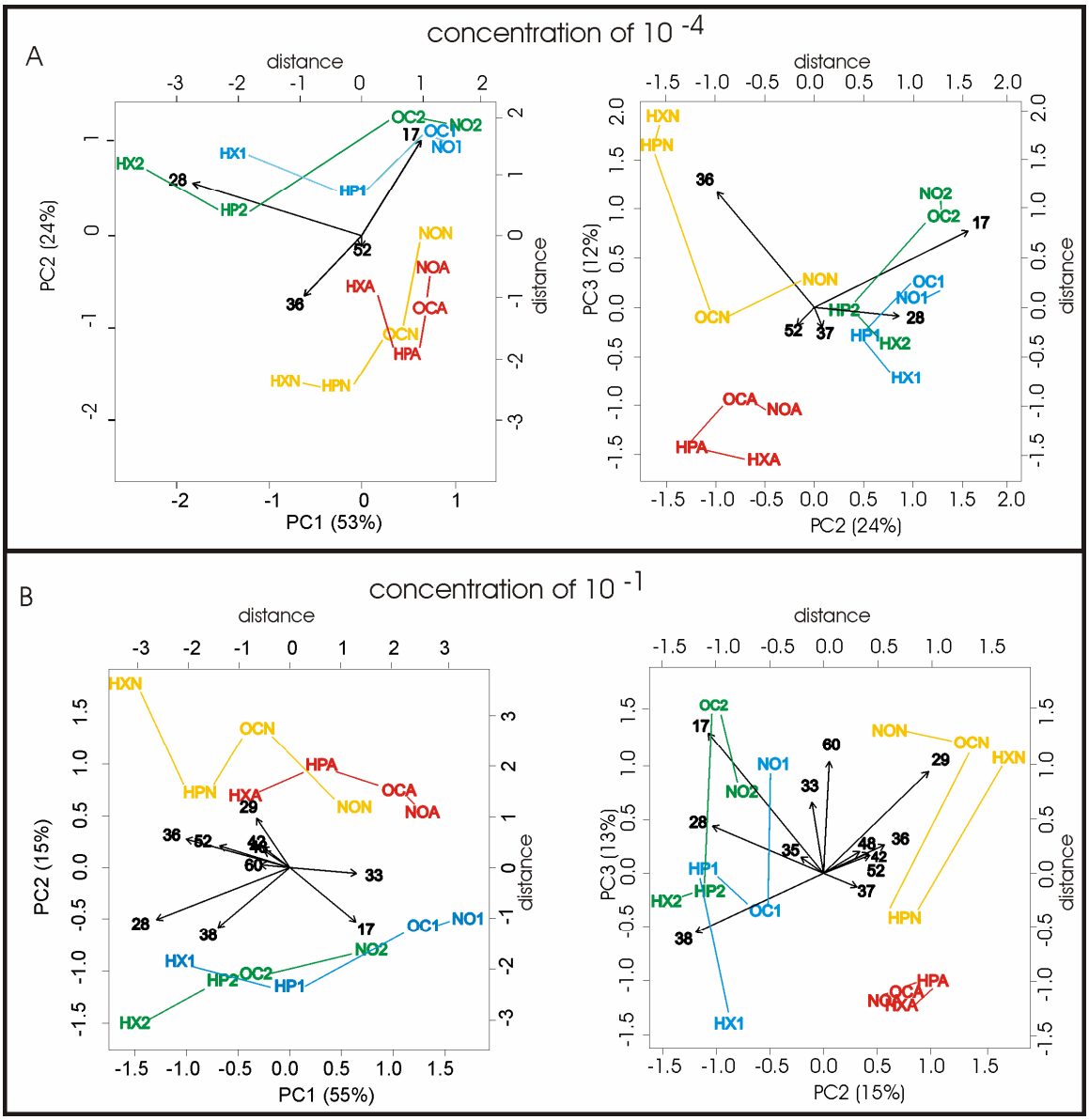

Figure 9) Principal component biplot of the responses to odors presented at A)10^{-4} and B) 10^{-1}. Lower and left axes depict the according PCs, scaled to unit variance. Upper and right axes show the mahalanobis distance between odor representations. Vector direction shows glomerular odor preference, vector length the standard deviation of glomerular activity across odors, eg. the relative contribution to separating the odors in space. Odors are colored according to their functional groups (primary alcohols: blue, secondary alcohols: green, aldehydes: red, ketones: orange). Odor abbreviations: HX1: 1-hexanol, HX2: 2-hexanol, HXA: hexanal, HXN: 2-hexanone, HP1: 1-heptanol, HP2: 2-heptanol, HPA: heptanal, HPN: 2-heptanone, OC1: 1-octanol, OC2: 2-octanol, OCA: octanal, OCN: 2-octanone, NO1: 1-nonanol, NO2: 2-nonanol, NOA: nonanal and NON: 2-nonanone.

the odors in the principal component space was mainly caused by the differential activity of three glomeruli (17, 28 and 36). PC1 was mainly affected by the decreasing response strength of glomeruli 28 and 36 and the increasing response strength of 17 when presented with odors of increasing carbon chain lengths. The separation of primary and secondary alcohols from aldehydes and ketones in PC2 was mainly accomplished by the much stronger responses of 17 and 28 to alcohols. The preferential activity of glomerulus 36 to ketones over aldehydes, in turn, separated these two functional groups in PC3. The same applies to the higher concentrations although more glomeruli contribute to the principal component space.

Similarity between glomerular activity patterns correlates strongly with perceptual odor similarity

The fact that the physiological responses to odors can be separated according to their chemical properties in a principal component space does not necessarily mean that this is relevant for the animal. Therefore a direct comparison to animal behaviour was necessary. Guerrieri and co workers measured the degree of generalization to the same 16 odors after having trained the animals to one of them, using a proboscis extension paradigm (PER). As a result, they published a perceptual similarity matrix based on the same odors we used in our study (Guerrieri *et al.*, 2005).

We therefore tested to what extent the similarities in odor evoked glomerular activity patterns were comparable to the perceptual similarities for these 16 odors. *Guerrieri et al.* also applied a PCA to their data and obtained a very similar separation of the odors in the principal component space.

To further investigate this correspondence, we calculated the Euclidean distances between the different glomerular odor representations for each frame measured and obtained a set of 40 distance matrices for each concentration. Each of these matrices was correlated with the perceptual similarity matrix (Figure 10). All 4 concentrations did correlate very well with the behaviour, and the extent of this correlation was

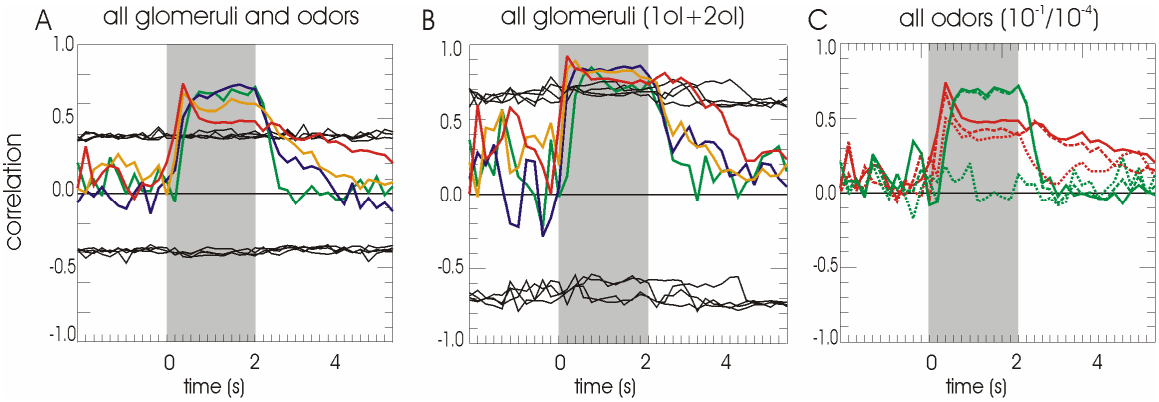

Figure 10) Correlation between physiological data and behavior, plotted over time. Different colors show different odor concentrations. red: 10^{-1}, orange: 10^{-2}, blue: 10^{-3}, green: 10^{-4}. Thin black lines depict the minimal and maximal correlation values obtained with the Monte Carlo analysis (See Methods). A) Physiological data based on all glomeruli and odors. B) Physiological data based on all glomeruli, but only primary and secondary alcohols as odors. C) Physiological data based on all odors, concentrations are 10^{-1} and 10^{-4}. Solid lines: all measured glomeruli are used to calculate the correlation, dashed lines: only the best five glomeruli (17, 28, 33, 36 and 52), dotted lines: without the best five glomeruli.

concentration independent (Figure 10A). Before stimulus onset, the correlations of all 4 concentrations were around or slightly above zero. Upon stimulation, the correlation values instantly increased. For the two higher odor concentrations, a peak correlation of r=0.73 (10^{-1}) and r=0.67 (10^{-2}) was reached already after 400 ms and then decreased to a lower level until stimulus offset. While for odors presented at the highest concentration the correlation values did not return to baseline before the end of the measurements, the values for 10^{-2} remained on a plateau until stimulus offset and then decreased to baseline. The correlation values of the two lower concentrations (10^{-3} and 10^{-4}) over time exhibited a different shape. Their increase upon stimulation was not quite as fast as for the higher concentrations, and instead of a distinctive peak, they reached a plateau on which they remained during stimulation. The level of this plateau was about as high as the maximal correlation values for the higher concentrations, with maximal values of r=0.73 for 10^{-3} at 1800 ms and r=0.71 for 10^{-4} at 2200 ms after stimulus onset. After stimulus offset, the correlation values decreased. Upon stimulus onset, the correlations for higher concentrations increased slightly faster, while those for lower concentrations decreased

faster following stimulus offset. For all four odor concentrations, the correlation values were highly significant (see Material&Methods).

We repeated the analysis limiting our data to the primary and secondary alcohols, as done by *Guerrieri et al.* (Figure 10B). Once more it became visible that our physiological data correlated extremely well with the behavioural data independently of concentration. The maximal correlation values for the 4 different odor concentrations were r=0.93 (10^{-1}), r=0.89 (10^{-2}), r=0.86 (10^{-3}) and r=0.85 (10^{-4}). Already before stimulus onset, the correlation values for the different odor concentrations fluctuated strongly, in the case of 10^{-1} even reaching r=0.69. The temporal characteristics of the correlations were similar to the ones observed when using all 16 odors. Upon stimulus onset the correlations quickly rose to their maximum. While 10^{-1} reached its maximum already 200 ms after stimulus onset, the correlations of 10^{-2} again reached their maximum another 200 ms later. This time, the correlations of the highest two concentrations decreased less during the remaining stimulus duration. For all except for the highest concentration, the correlation values decreased quickly after stimulus offset. The significance threshold, as defined by the maxima of the Monte Carlo analysis was much higher than when including all odors into the analysis, suggesting that the high correlation obtained from the alcohols has to be interpreted with caution.

These results show that the similarity between odors, as calculated by the Euclidean distances between their respective glomerular activity patterns, is predictive of the odor similarity perceived by the animals.

The glomerular activity redundantly encodes odor identity

To elucidate which glomeruli were responsible for this high degree of correlation, we repeated this analysis excluding specific glomeruli from our physiological data before calculating the Euclidean distance matrices (Figure 10C). First we excluded all but the 5 most sensitive glomeruli, identified as 17, 28, 33, 36 and 52 in the PCA above. When correlating the resulting distance matrix of 10^{-4} with the perceptual matrix the correlation values and the temporal characteristics remained unchanged (r=0.70 as compared to r=0.71 for all glomeruli). To the contrary, when excluding especially these five glomeruli from our data, the correlation values of the lowest concentration collapsed to r=0.24. We

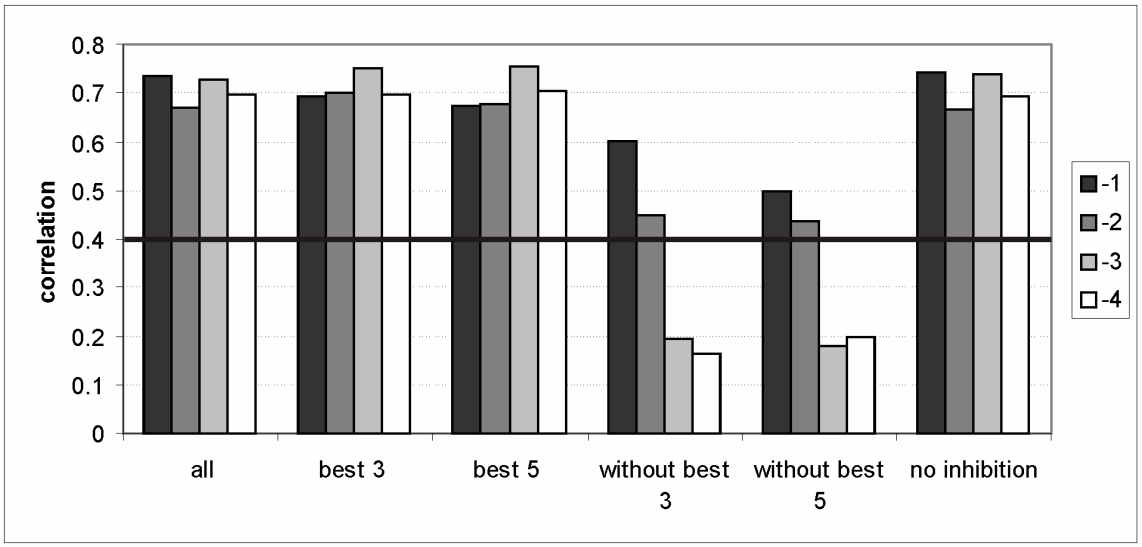

Figure 11) Maximal correlation between physiology and behavior, including different sets of glomeruli. Similar procedure as in Fig 9C). *all*: all 14 glomeruli are included; *best3*: only best three are included (17, 28, 36); *best5*: best three glomeruli plus T1-33 and T1-52 are included; *without best3* and *withoutbest5*: calculations include all but the best three or five glomeruli; *no inhibition*: glomerular activity values below zero are set to zero before correlation. Horizontal line approximates the maxima of the Monte Carlo distributions.

repeated the analysis for the concentration of 10^{-1}. The matrix based on only the five glomeruli again correlated very well with the behavioural matrix (r=0.67 as compared to r=0.73 for all glomeruli). When excluding these glomeruli, in turn, this time the correlation values remained relatively high (r=0.50). The increase upon stimulation was delayed and the correlation values decreased instantly upon stimulus offset.

We further investigated this differential effect of missing glomeruli by excluding different sets of glomeruli from the analysis before calculating the correlation (Figure 11). In Figure 9A), we had seen that three glomeruli had a strong effect on the positions of the odors in the principal component space. We tested the correlations based only on these three glomeruli, namely 17, 28 and 36, and after excluding them from our data. Their presence alone was sufficient to maintain all correlations at high level (r=0.7, r=0.7, r=0.75, r=0.7 for concentrations from 10^{-1} to 10^{-4}).

Removing these three glomeruli from the data before the analysis, in turn, had a strong deteriorating effect. While at higher concentrations the present glomeruli were somehow sufficient to rescue the correlation, the correlation values for the lower

concentrations collapsed below the significance boundary of 0.4 established by the Monte Carlo analysis (r=0.6, 0.45, r=0.2, r=0.16 for concentrations from 10^{-1} to 10^{-4}). Additionally excluding 33 and 52 did further decrease the correlation value for the highest concentration of 10^{-1}.

As the role of the glomeruli which decreased their activity during stimulation remained enigmatic, we tested their impact on our correlation values. Prior to generating the Euclidean distance matrix, we "inactivated" them, by setting their responses to zero. No considerable effect on the strength of correlation could be observed (r=0.74, r=0.67, r=0.74, r=0.7 for concentrations from 10^{-1} to 10^{-4}).

As a result, we conclude that at high concentrations odors are encoded redundantly in the glomerular PN activity. Even though the correlations were best when all glomeruli were present, the absence of one or several glomeruli could be compensated for by the remaining ones.