Sparassizing and Temporal Sharpening of Olfactory Representations in the Honeybee Mushroom Bodies

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INTRODUCTION

The main olfactory brain regions in insects are the antennal lobes (AL) and the mushroom bodies (MB; Fig. 1A). The AL is the first synaptic processing station and a functional homologue to the vertebrate olfactory bulb (Hildebrand and Shepherd 1997). The AL of honeybees consists of ~160 subcompartments, called glomeruli (Flanagan and Mercer 1989; Galizia et al. 1999). Projection neurons (PN) convey the output from the AL glomeruli and their boutons at the KC synapse. To reveal transformations taking place in the postsynaptic KCs, we recorded odor-evoked responses in KCs and compared them to their presynaptic input from PNs.

At all three processing stages, odors reliably evoke combinatorial activity patterns. However, in contrast to PNs, KCs code odors in a sparse way and generate only brief responses at stimulus onset. We found that KC’s high odor-specificity originates at two steps: first, in a presynaptic sharpening of PN synaptic output and second, in a sparsening in KC response. Interestingly, the temporal sharpening of KCs’ responses is established only at the postsynaptic side. Our results also show that PN activity generated within the first 200 ms determine whether a KC will respond.

METHODS

In vivo bee preparation and dye loading

Experiments were performed with forager bees, Apis mellifera carnica. Bees were collected at the hives, chilled, and fixed as a whole

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animal into recording chambers. To prevent visual stimulation through light exposure during optical recordings, the complex eyes were covered with dental wax mixed with charcoal. To allow access to the brain, the head capsule was opened by removing a piece of cuticle surrounding the antennae, the ocelli, and the complex eyes. Where necessary, pieces of trachea covering the injection sites were removed. Neurons were stained with dextran-conjugated Ca\(^{2+}\) indicators by mass-injection (Fura-2 dextran, Calcium Green-1 dextran, Molecular Probes, Eugene, OR). Injection needles were pulled from 1-mm glass capillaries to a tip diameter of \(\sim 10\) \(\mu\)m and were coated with dye dissolved in 2% solution of bovine serum albumin. PN dendrites in the AL were retrogradely stained with Fura-2 dextran via their axons running through the l-ACT, PN axon terminals were anterogradely stained with either Fura-2 dextran or Calcium Green-1 dextran via l-ACT PN soma clusters in the dorso-medial part of the AL and dendrites and somata of clawed KCs were retrogradely stained by injecting Fura-2 dextran into the clawed KC axons located in the ventral part of the vertical lobes (see Fig. 1). Application of Ca\(^{2+}\) indicators with different fluorescence spectra for clawed KC and PN bouton staining allowed simultaneous recording of clawed KCs and PN boutons. After dye injection, the head capsule was closed with the cuticle piece and sealed with n-eicosan (Sigma). Half an hour later, bees were fed until satiation and kept in a container at 17–20°C for 8–24 h. To prevent movement artifacts, abdomen and legs were immobilized with dental wax, muscles which innervate the antennae were carefully removed, the mouthparts were truncated and the esophagus was taken out. Immediately afterward, the head capsule was washed with bee Ringer (which contained, in mM: 130 NaCl, 7 CaCl\(_2\), 6 KCl, 2 MgCl\(_2\), 160 sucrose, 25 glucose, and 10 HEPES, pH 6.7, 500 mosmol). To stabilize the brain, a 1.5% solution of low-melting agarose (Sigma, A2576) was injected into the head capsule. Experiments started 30 min after preparation.

**Odor stimulation and imaging.**

We used the following odors: 1-hexanol, 2-octanol, limonene, linalool (all from Sigma), and peppermint oil (local drugstore). Odors were diluted in mineral oil to adjust for differences in vapor pressure (1-hexanol, 16.2%; 2-octanol, 60%; limonene, 9%; and linalool, 46.2%). Odor solution (4 \(\mu\)l) was applied onto a 2 cm\(^2\) piece of filter paper and placed in a plastic syringe. Using a computer controlled olfactometer (Galizia et al. 1997), odors were injected into a continuous air stream which was directed to both antennae. Odors were presented as 3-s pulses three or six times in a pseudo-randomized order with a 1-min interstimulus interval.

Images were acquired at room temperature with a sampling rate of 5 Hz using a TILL-Photonics imaging set up mounted on a fluorescence microscope (Olympus BX-50WI). Measurements started 2 s before stimulus onset and lasted for 12 s. In the AL, l-ACT PN dendrites were recorded through a \(\times 20\), NA = 0.95 Olympus dip objective. Fura-2 was excited at a single wavelength (390 nm) to reduce photodamage. Fluorescence was detected through a 410-nm dichroic mirror and a 515-nm LP filter. The MB calyx was illuminated alternately with 390 and 475 nm at rate of 10 Hz; the fluorescence was detected through a Omega 505DRLPX filter. For imaging KCs and PN boutons were recorded through a \(\times 60\), 0.9 W Olympus objective with an Imago QUE CCD camera (1,376 \times 1,040 pixels, 8 \times binned on chip to 172 \times 130). In the median MB calyx, KCs and PN boutons were recorded through a \(\times 60\), 0.9 W Olympus objective with an Imago QUE CCD camera (640 \times 480 pixels, 4 \times binned on chip to 160 \times 120). The spatial resolution was 1.3 \(\mu\)m/pixel and allowed resolving single l-ACT PN boutons and clawed KC dendrites and somata. Fura-2 measurements of clawed KCs and PN boutons were carried out with the same filter settings used for l-ACT PN dendrite recordings. For simultaneous recording of clawed KCs (Fura-2) and l-ACT PN boutons (Calcium Green-1) the MB calyx was illuminated alternately with 390 and 475 nm at rate of 10 Hz; the fluorescence was detected through a Omega 505DRLPX filter.

**Data analysis.**

Data were analyzed using custom-written programs in IDL (RSI, Boulder, CO). Morphological images were unsharp mask filtered in Photoshop (Adobe). Ca\(^{2+}\) signals were calculated as fluorescence change relative to background fluorescence (\(\Delta F/F\)). Background fluorescence (\(F\)) was determined by an average of five frames obtained before stimulation and was subtracted from every frame of a measurement to give \(\Delta F\). Signals were corrected for dye bleaching by subtracting a logarithmic curve fitted to the mean brightness decay of the entire image frames, excluding frames during the stimulus. Because the same value was subtracted from each pixel within a frame, the bleach correction did not affect the spatial activity pattern of the Ca\(^{2+}\) signals (Galizia and Vetter 2004). Signals of Fura-2 have been inverted because it decreases its fluorescence at 390-nm excitation light in response to increasing Ca\(^{2+}\) concentrations. Activity patterns...
are depicted as color-coded images, representing the averaged $\Delta F/F$ values of 15 frames (3 s) during the odor stimulus. For better visualization a spatial low-pass filter ($3 \times 3$ pixels) was applied onto the images. To analyze the response dynamics, activity patches, corresponding to responsive PN dendrites, PN boutons, KC dendrites, and KC somata during the 3-s odor pulse, were selected. These pixels were averaged without filtering. The time courses obtained were then analyzed in Excel (Microsoft). For analyzing the dynamics of Ca$^{2+}$ signals, three measurements were averaged. Noise was defined pixel wise as the SD of the fluorescence values over a 2-s interval (10 frames) before odor stimulus. To determine the proportion of excitatory and inhibitory responses, only signals with amplitudes $>$5 times noise were analyzed. To determine the onset of responses only, signals 7 times above noise were analyzed. The response onset was defined as signal $>$3 times noise.

Correlation analysis of spatial activity patterns was performed by computing the linear correlation between them. To determine the response profiles of PN dendrites, boutons and KC somata, three measurements were averaged. Because KCs respond within 600 ms after the stimulus onset (Fig. 6B), only responses occurring within the first three frames (600 ms) after stimulus onset were considered for PNs and KCs, to achieve comparability. Signals were considered as responses if the averaged signal during the first three frames was three times greater than noise. Isolated activity spots smaller than $2 \times 2$ pixels (2.6 $\mu m^2$) were regarded as noise and rejected. To detect responses of individual KC somata or PN boutons, scattered light was reduced by applying an unsharp mask filter (Galizia and Vetter 2004). For unsharp mask-filtering images were low-pass filtered (30 nm Fura signal). Responses were phasic-tonic and/or complex, Signal size ranged from 0.5 to 8% (2.41 $\pm$ 0.02% $\Delta F/F$ of 390 nm Fura signal). Responses were phasic-tonic and/or complex, which corresponds to the temporal dynamics observed for intracellulary recorded spike rates (Abel et al. 2001; Galizia and Krimmerle 2004; Müller et al. 2002) (Fig. 2C). Of the 27 glomeruli accessible from the frontal aspect, 5.3 $\pm$ 0.7 were activated by any of the four odors presented, resulting in a glomerular response probability of $p_G = 0.20 \pm 0.02$ per PN and odor.

Response properties of l-ACT PN boutons (AL glomeruli)

Dendritic Ca$^{2+}$ signals of PNs were recorded in the AL glomeruli (Fig. 2A). Previous studies have shown that glomerular Ca$^{2+}$ transients correlate with the action potential activity of PNs, which arborize in the same glomerulus (Galizia and Krimmerle 2004). Therefore we considered the glomerular Ca$^{2+}$ signal of PN dendrites as a monitor for the activity of individual PNs at the output side of the glomerulus.

We measured signals from l-ACT PNs. These neurons are uniglomerular and receive afferent input from 1 of $\sim$70 glomeruli on the dorsorostral part of the AL (Flanagan and Mercer 1989; Galizia et al. 1999). On the average $27 \pm 0.5$ (means $\pm$ SE) of these 70 glomeruli were visible in each animal (Fig. 2A). A total of 370 glomeruli from 14 bees were imaged. As previously shown (Sachse and Galizia 2002), PNs showed background activities in the absence of olfactory stimulation. Odor pulses evoked combinatorial activity patterns in PN dendrites across glomeruli (Fig. 2B). Intracellular calcium also increased in their somata. Most of the dendritic PN responses were excitatory (98.5%), but some (1.5%) were inhibitory. Signal size ranged from 0.5 to 8% ($2.41 \pm 0.02\% \Delta F/F$ of 390 nm Fura signal). Responses were phasic-tonic and/or complex, which corresponds to the temporal dynamics observed for intracellulary recorded spike rates (Abel et al. 2001; Galizia and Krimmerle 2004; Müller et al. 2002) (Fig. 2C). Of the 27 glomeruli accessible from the frontal aspect, 5.3 $\pm$ 0.7 were activated by any of the four odors presented, resulting in a glomerular response probability of $p_G = 0.20 \pm 0.02$ per PN and odor.

Response properties of l-ACT PN boutons (MB calyx)

To investigate the relationship between AL output and MB input carried by PNs, we next examined the response properties of PN boutons in the MB calyx. PN boutons were selectively filled with the Ca$^{2+}$ indicator via l-ACT PN soma clusters located on the dorsomedial surface of the AL (Fig. 2A). We recorded from a total of 105 responsive boutons (9 bees) in the frontal lip area of the median calyx. Odor-induced responses consisted of isolated activity spots, which were colorized with boutons visible in the raw fluorescence image (Fig. 2D). A total of 87.2% of the responses were excitatory, with fluorescence changes ranging from 0.3 to 3.8% (0.87 $\pm$ 0.03% $\Delta F/F$), whereas the remaining 12.8% of the responses were inhibitory. As is the case in the AL, stimulation with odors activated odor-specific ensembles of PN boutons (Fig. 2E). Boutons were located at different focal depths and were not always visible in the raw fluorescence image, so we did not determine their response probability.

The response dynamics of PN boutons resembled those of PN dendrites (glomeruli). They showed phasic-tonic and/or complex time courses and often outlasted the odor pulse by several seconds (Fig. 2F). The across-odor response profile of the PN boutons also resembled the one observed in PN dendrites (Fig. 3): Limonene odor elicited fewer responses than the
other three tested odors, and glomeruli and boutons responding to 2-octanol often also responded to 1-hexanol. However, in boutons the proportion of inhibitory responses was higher than in glomeruli (12.8 vs. 1.5%).

**Response properties of clawed KC (MB calyx)**

In the MB calyx, L-ACT PNs synapse onto clawed KC, a population of cells intrinsic to the MBs. To investigate how odor information is processed within the MB, we therefore...
recorded from clawed KCs. Clawed KCs can be reliably identified based on their anatomy (Fig. 4, A and B). They differ from other KC types in that they feature a columnar arrangement of their unbranched dendrites, which possess 5–10 claw-like synaptic structures. Their somata (3–7 μm in diameter) are located outside the calyx region rostral to the lip and are connected to the dendrites through a 10- to 100-μm-long primary neurite. Dendrites and somata of these cells were selectively stained by injecting Fura-2 dextran into their axons in the ventral part of the vertical lobe. Dendritic and somatic responses to odors were then recorded in the lip region of the MB calyx. Figure 4B shows an example where only one clawed KC was stained. Its soma and dendritic branches were visible in the fluorescence image. The time courses of Ca\(^{2+}\) transients in dendrite and soma were similar. Both followed a uniform phasic uprise at stimulus onset with off responses after the end of the odor stimulus. However, Ca\(^{2+}\) signal decay was slower in the soma.

In the soma layer, odors induced sharp activity peaks in individual clawed KC somata, which showed fluorescence changes ranging from 0.3 to 2.7% (1.09 ± 0.03 Δ\(F/F\), 67 responses in 8 bees). In the lip neuropil of the calyx, odor-induced dendritic activity appeared in larger, less well-defined regions and showed fluorescence changes ranging from 0.5 to 6% (1.49 ± 0.03% Δ\(F/F\), 473 responses in 15 bees). Those activity patches had a columnar or elongated shape of 20–60 μm in diameter, which matches the morphology of the dendritic trees of single-clawed KCs (Fig. 4, B and D). Dendritic activity patches and activated somata often occurred close to each other, suggesting that they belong to the same clawed KC. Response dynamics were common among clawed KCs. Figure 4C shows 10 representative dendritic-clawed KC responses. Clawed KC exclusively exhibited excitatory responses. Off responses were visible in 40% of the measurements.

Different odors induced different patterns of activated clawed KC dendrites with different degrees of overlap, whereas stimulation with the same odor reliably activated the same clawed KC ensemble (Fig. 4D). To confirm the odor specificity of the spatial response patterns, the correlation coefficient between dendritic activity patterns was calculated as a measure of similarity. The correlation coefficients were generally low, something that can be attributed to the fact that only a small surface of the imaged area was activated by any one stimulus, so that correlation coefficients were strongly influenced by the noise in the nonresponding areas. The mean correlation coefficient between responses that have been elicited by repeated presentation of the same odor was always higher than between responses to different odors (0.37 ± 0.04 vs. 0.14 ± 0.04, Wilcoxon signed-rank test, \(P < 0.001, n = 15\); Fig. 4E).

Due to the limited spatial resolution of the signal, it was not possible to determine whether the overlap between the dendritic activity patches reflects responses from the same clawed KC or from clawed KCs with anatomically overlapping dendritic trees. However, we found that the responses in soma mirrored the activity of their corresponding dendrites. Therefore we quantified the response properties of individual clawed KCs by analyzing the activity of soma as a monitor for individual KCs. We recorded from 1680 clawed KC somata in 12 animals, which were exposed to all four odors. Seventy-seven somata responded to at least one of the four tested odors. Odors activated nonoverlapping clawed KC ensembles. The response probability of a given clawed KC was \(p_{KC} = 0.013 ± 0.001\) (means ± SE) per odor.

**Sparsening of the population code in the MB**

To analyze the transformation of odor representations in the MB and to evaluate the contribution of pre- and postsynaptic processing, we compared the responses of PN dendrites, PN boutons, and clawed KCs to the four tested odors (1-hexanol, 2-octanol, limonene, and linalool). We first asked whether odor processing along the olfactory pathway leads to a change in the sparseness of the population code. The term “sparseness” describes the proportion of active units at any time (population sparseness) and/or the mean tuning width of each neuron.
A sparse code is characterized by few neurons active at any time and/or a narrow tuning width (Olshausen and Field 2004; Willmore and Tolhurst 2001). Figure 5 illustrates the progressive sparsening of the odor code from PN dendrites to PN boutons and to clawed KC somata. In the AL, PNs were broadly tuned because many glomeruli respond to more than one odor. PN boutons were more narrowly tuned, as the histogram was skewed toward a lower number of odors. Clawed KCs showed the highest sparseness, were extremely odor specific, and responded mostly to one odor only. This result demonstrates that odor processing in the MB results in a sparsening of the odor code carried by clawed KCs. Furthermore it indicates that sparseness of the population code occurs not only at the level of the postsynaptic clawed KCs but, to a lower extent, also at the presynaptic terminals of the PNs. Thus the high degree of sparseness at the KC level can be attributed to at least two distinct processes.

Change in response dynamics

Understanding the logic of sensory information coding requires the identification of those features of the neural representations that are read out by downstream neurons. We therefore tried to narrow down features of the PN responses that might be read out by clawed KCs. Odor-evoked activity patterns of PNs are not stationary, but show temporal modulations, which evolve over time and lead to activity patterns that in several species are most odor-specific after some hundreds of milliseconds following odor onset (bee: Galán et al. 2004; Galizia et al. 2000; locust: Stopfer et al. 2003; zebrafish olfactory bulb: Friedrich and Laurent 2001). It has been suggested that downstream neurons have to read out these temporal patterns to retrieve the odor information. If that is true, then the temporal patterns of PN responses must be relevant for KCs, and KCs tuned to PN activity patterns should respond with time lags corresponding to particular phases of the dynamic response.
was temporally complex during an odor stimulus of 3 s and often outlasted the odor stimulus, responses in KCs were always limited to brief phasic responses. These results show that the onset of KCs responses occurs within <200 ms of the initial PN responses, whereas later input does not activate KCs. The results also demonstrate that the temporal sharpening of the KC response reflects postsynaptic processing because it is not present in PN boutons.

Figure 7 elucidates our findings and illustrates a proposed mechanism that may mediate the transformation of odor representations along the olfactory path (see DISCUSSION): Within the AL, odors evoke dense across-fiber patterns of l-ACT PNs. The spatial activity pattern of the presynaptic terminals of these projection neurons is sparsened within the MB lip—presumably through reciprocal microcircuits between GABAergic neurons and l-ACT PNs. Clawed KCs integrate l-ACT PN activity within 200 ms and transform the complex temporal pattern into brief phasic responses. The sharpening of clawed KCs’ temporal responses may be mediated by a more global inhibition via GABAergic feedback neurons. Moreover the PN-to-KC divergence together with a high spiking threshold may lead to a further sparsening of clawed KC population responses.

To test this hypothesis, we investigated the temporal relationship between PN and clawed KC responses by simultaneously recording from PN boutons and clawed KC using two different dyes. The traces in Fig. 6A show Ca$^{2+}$ transients measured at 5 Hz in individual PN boutons and colocalized KC dendrites. There was no detectable time lag between the responses of PN boutons and clawed KCs, indicating that the delay of transmission was well below the 200-ms cycle duration in the recordings. To get a more accurate estimation of the delay between PN boutons and KC responses, we compared response onsets to 3-s odor pulses. Only animals with signal/noise ratios >5 were chosen, and three measurements were averaged (PNs: 3 times 655 measurements in 11 bees; KCs: 3 times 184 measurements in 15 bees).

Most of the PNs responded with constant delays. 90% of the responses occurred within 400 ms after stimulus onset, whereas the remaining 10% showed delays of ≤1,000 ms. Ninety-five percent of the KC responses occurred within 400 ms after stimulus onset, and the remaining 5% occurred within 400–600 ms. No KC responded at a later time point. Figure 6B shows the time courses of Ca$^{2+}$ signals in PN dendrites, boutons, and KCs and illustrates the relationship between the response dynamics of PNs and KCs. Even though PN activity

**FIG. 5.** Progressive sparsening of population responses in the MB. The histograms show the percentage of responsive units (PN dendrites measured in the glomeruli, PN boutons, and clawed KC somata) that were activated by 1, 2, or more odors (4 odors were presented) The distributions varied significantly between the different processing stages and showed a progressive increase in the sparseness of odor representations from PN dendrites to PN boutons and to clawed KCs (χ² test, applied on absolute numbers of responsive units, P < 0.001; PN dendrites: n = 298 responsive glomeruli in 14 bees; PN boutons: 90 responsive in 9 bees; clawed KCs: 77 responsive somata in 12 bees).
We confined the area monitored in the MB to the frontal part of the lip for technical reasons. We assume that the properties of PN boutons and clawed KCs located in this area are representative for any other part of the MB lip. Within the MB calyx, KCs of different types and sensory modalities are arranged in concentric layers. Olfactory KCs are homogeneously distributed along the lip layer, where they receive input from the l-ACT PN boutons (Mobbs 1982). Although l-ACT PN boutons are not homogeneously distributed (Müller et al. 2002), the recorded area was large enough to contain several boutons of each PN.

Besides l-ACT PNs recorded in this study, m-ACT PNs also project onto the lip area. m-ACT PNs receive input from an area in the AL that was not accessible for our imaging technique. Müller et al. (2002) found that m- and l-ACT PNs differ in their response properties. Unlike l-ACT PNs, m-ACT PNs show odor-specific response latencies of several 100 ms. Because clawed KCs exhibit stable and immediate responses, we conclude that the recorded responses of clawed KCs are exclusively driven by l-ACT PNs. In the same area where we recorded from clawed KCs, there are other KCs (Type 1 KCs) that might be the preferential targets for m-ACT PNs. It is likely that such KCs receiving input from m-ACT PNs have very different response properties from the clawed KCs described here, but these KCs were not filled with dye and did not contribute to the recorded signal.

**Transformation of odor coding within l-ACT PNs: dendrites to boutons**

Within the MB lip, PN boutons convey excitatory input to KCs and GABAergic neurons, which in turn provide inhibitory feedback onto the PN boutons themselves (Fig. 1B) (Ganeshina and Menzel 2001). To assess the role of these microcircuits for odor processing, we compared the odor responses in PN dendrites within the AL glomeruli with those at the PN boutons.
in the MBs (Figs. 2 and 3). Both PN dendrites and boutons exhibited reliable combinatorial patterns of odor-evoked activity and showed similar response profiles. However, PN dendrites responded less frequently with inhibition than their boutons (1.5 vs. 12.8%) and were more broadly tuned to odors. Afferent PN activities only at the beginning of each response bout. However, it cannot be excluded that action potentials that might be generated by clawed KCs after the end of the odor stimulus, some clawed KCs’ response onset was restricted to the beginning of PNs’ response and followed a phasic time course (Fig. 6). There were no clawed KCs that responded at a later time point. This indicates that clawed KCs might indeed receive delayed odor-driven inhibition that lasts as long as the odor stimulus and prevents them from spiking again. Candidate neurons for the presumed odor-driven delayed inhibition of clawed KCs are GABAergic feedback neurons, which receive input from a large population of KCs in the MB pedunculus and lobes, presumably leaving out the clawed KCs, and project back to the lip of the MB (Grünewald 1999a; Schäfer and Bicker 1986). These neurons exhibit phasic-rectified responses that usually do not exceed stimulus duration (Grünewald 1999b). Thus the off responses of clawed KCs

Transformation from l-ACT PNs to clawed KCs

SPARSENING. We have analyzed the response characteristics of l-ACT PNs and clawed KCs across their population. In both, odors are coded in form of combinatorial activity patterns. However, clawed KCs with the same olfactory profile are not grouped into functional subunits as is the case for the PNs in the AL, where they all coalesce in one glomerulus. We demonstrated that odor-activated clawed KC ensembles are sparse compared with PN ensembles. Unlike PNs, clawed KCs responded very selectively to odors. The observed response probability \( p_{KC} \) of a given clawed KC averages 0.013 per odor and is significantly lower than the response probability of PN dendrites \( (p_G = 0.2) \). The observed sparseness of the recorded clawed KCs is in accordance with the finding of a sparse code in KCs of locusts and Drosophila (Perez-Orive et al. 2002; Wang et al. 2004) and may therefore represent a general property of KCs. Both the distributive character and the sparseness of the clawed KC code may be a result of the divergent-convergent connectivity between PNs and clawed KCs (Laurent 2002). About 500 l-ACT PNs converge onto estimated 20,000 clawed KCs (counted and interpolated from Golgi stains, J. Rybak, personal communication). Knowing that each l-ACT PN has \( \sim 400 \) boutons (Müller et al. 2002), this yields a ratio of \( \sim 10 \) boutons per clawed KC. This matches the number of claw-like dendritic specializations in each clawed KC. Assuming that each of these claws contacts a different PN, how many of these PN inputs must be coactive to induce a clawed KC response? With a response probability \( p_G = 0.2 \) in PNs, a cumulative probability corresponding to clawed KC activity of \( p_{KC} = 0.013 \) is reached when 5.8 or more of 10 PNs are simultaneously active. Thus a single clawed KC would compress information carried by a subset of 6–10 PNs. This means that clawed KCs must have a high firing threshold, which could be the result of the intrinsic properties of KCs and/or of odor-driven inhibition (Laurent and Naraghi 1994; Perez-Orive et al. 2002).

TEMPORAL SHARPENING. Clawed KCs had a phasic response with a fast onset of \(< 200 \) ms. This observation conflicts with studies in locusts. There odors evoke transient spike synchronizations between PNs which develop over hundreds of milliseconds after stimulus onset (Laurent and Davidowitz 1994; Laurent et al. 1996; Stopfer et al. 2003; Wehr and Laurent 1996) and lead to KCs that respond with variable, odor-specific latencies (Perez-Orive et al. 2002; Stopfer et al. 2003). The short and stable time-lag between responses in l-ACT PNs and clawed KCs in the bee suggests that clawed KCs decode afferent PN activities only at the beginning of each response bout. However, it cannot be excluded that action potentials that might be generated by clawed KCs after the 200 ms may contain information that we could not measure with Ca\(^{2+}\) imaging.

What are the mechanisms underlying the short integration time and the sharp response dynamics of clawed KCs? We assume that both result from odor-driven delayed inhibition of clawed KCs. Odor-driven inhibitory input was found in locust KCs (Laurent and Naraghi 1994; Perez-Orive et al. 2002). The response dynamics of bees’ clawed KCs provide clear evidence that they might receive inhibitory input as well. Despite continuous input from PNs, clawed KCs’ response onset was restricted to the beginning of PNs’ response and followed a phasic time course (Fig. 6B). There were no clawed KCs that responded at a later time point. This indicates that clawed KCs might indeed receive delayed odor-driven inhibition that lasts as long as the odor stimulus and prevents them from spiking again. Candidate neurons for the presumed odor-driven delayed inhibition of KCs are GABAergic feedback neurons, which receive input from a large population of KCs in the MB pedunculus and lobes, presumably leaving out the clawed KCs, and project back to the lip of the MB (Grünewald 1999a; Schäfer and Bicker 1986). These neurons exhibit phasic-rectified odor responses that usually do not exceed stimulus duration (Grünewald 1999b). Thus the off responses of clawed KCs

FIG. 7. Model of odor processing in the MB input. l-ACT PNs (green) convey odor information in form of dense across-fiber patterns of action potential bursts. In the MB input, they make excitatory synapses with clawed KCs (red) and with terminals of extrinsic GABAergic neurons (dark blue), which, in turn, make inhibitory synapses with PN boutons. The inhibitory input onto PN boutons has the effect that not all boutons of an excited PN will respond, resulting in a sharpening of the response profiles of PN boutons. GABAergic feedback neurons (light blue), which receive input from a large number of KCs (presumably excluding clawed KCs), form a global inhibitory loop, and mediate delayed, odor-driven inhibition of clawed KCs. The inhibitory input onto clawed KCs restricts their integration time window for PN input to <200 ms and shortens their response duration. After the end of the odor stimulus, the inhibitory input declines and clawed KCs are released from inhibition and become excitable again. Because many PNs continue spiking after the end of the odor stimulus, some clawed KCs exhibit off responses. The divergent connectivity between l-ACT PNs and clawed KCs, together with a high spiking threshold of clawed KCs, leads to a further sharpening of clawed KCs’ population response.

Transform from l-ACT PNs to clawed KCs

SPARSENING. We have analyzed the response characteristics of l-ACT PNs and clawed KCs across their population. In both, odors are coded in form of combinatorial activity patterns. However, clawed KCs with the same olfactory profile are not grouped into functional subunits as is the case for the PNs in the AL, where they all coalesce in one glomerulus. We demonstrated that odor-activated clawed KC ensembles are sparse compared with PN ensembles. Unlike PNs, clawed KCs responded very selectively to odors. The observed response probability \( p_{KC} \) of a given clawed KC averages 0.013 per odor and is significantly lower than the response probability of PN dendrites \( (p_G = 0.2) \). The observed sparseness of the recorded clawed KCs is in accordance with the finding of a sparse code in KCs of locusts and Drosophila (Perez-Orive et al. 2002; Wang et al. 2004) and may therefore represent a general property of KCs. Both the distributive character and the sparseness of the clawed KC code may be a result of the divergent-convergent connectivity between PNs and clawed KCs (Laurent 2002). About 500 l-ACT PNs converge onto estimated 20,000 clawed KCs (counted and interpolated from Golgi stains, J. Rybak, personal communication). Knowing that each l-ACT PN has \( \sim 400 \) boutons (Müller et al. 2002), this yields a ratio of \( \sim 10 \) boutons per clawed KC. This matches the number of claw-like dendritic specializations in each clawed KC. Assuming that each of these claws contacts a different PN, how many of these PN inputs must be coactive to induce a clawed KC response? With a response probability \( p_G = 0.2 \) in PNs, a cumulative probability corresponding to clawed KC activity of \( p_{KC} = 0.013 \) is reached when 5.8 or more of 10 PNs are simultaneously active. Thus a single clawed KC would compress information carried by a subset of 6–10 PNs. This means that clawed KCs must have a high firing threshold, which could be the result of the intrinsic properties of KCs and/or of odor-driven inhibition (Laurent and Naraghi 1994; Perez-Orive et al. 2002).
after the stimulus offset could reflect release from inhibition that makes them once again sensitive for input from PNs that remains active after stimulus offset.

Clawed KCs of the γ-lobe in Drosophila are particularly important for olfactory short-term memory (Zars et al. 2000). Anatomical similarities between clawed KCs in bees and Drosophila suggest they are homologues (Strausfeld 2002). Accordingly, the clawed KCs recorded in our study might play a major role in memory formation. Indeed the code used clawed KCs might be ideally suited for computations underlying odor learning. Each odor is encoded in a quasi-simultaneous combinatorial pattern of active clawed KCs without complex temporal activity sequences, which is an ideal substrate for associative learning rules of a Hebbian type (Lechner and Byrne 1998).

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REFERENCES


