Dynamics of Olfactory Bulb Input and Output Activity During Odor Stimulation in Zebrafish

Rainer W. Friedrich and Gilles Laurent

INTRODUCTION

Odors are first represented in the olfactory bulb (OB) by patterns of afferent activity across its input elements, the olfactory glomeruli, where axons of olfactory receptor neurons (ORNs) expressing the same odorant receptor type converge (Mombaerts 1999). A given glomerulus can respond to multiple odors, and a given odorant activates multiple glomeruli. Hence, odor identity is represented combinatorially by patterns of glomerular activation. Studies using optical imaging and single unit recordings from the input and output neurons, i.e., olfactory receptor neurons (ORNs) and mitral cells (MCs), respectively. A panel of 16 natural amino acid odors was used as stimuli. Responses of MCs, but not ORNs, changed profoundly during the first few hundred milliseconds after response onset. In MCs, but not ORNs, the total evoked excitatory activity in the population was initially odor-dependent but subsequently converged to a common level. Hence, the overall population activity is regulated by network interactions in the OB. The tuning widths of both ORN and MC response profiles were similar and, on average, stable over time. However, when analyzed for individual neurons, MC response profiles could sharpen (excitatory response to fewer odors) or broaden (excitatory response to more odors), whereas ORN response profiles remained nearly unchanged. Several observations indicate that dynamic inhibition plays an important role in this remodeling. Finally, the reliability of odor identification based on MC population activity patterns improved over time, whereas odor identification based on ORN activity patterns was most reliable early in the odor response. These results demonstrate that several properties of MC, but not ORN, activity change during the initial phase of the odor response with important consequences for odor-encoding activity patterns. Furthermore, our data indicate that inhibitory interactions in the OB are important in dynamically shaping the activity of OB output neurons.

Address for reprint requests and other correspondence: R. Friedrich, Max-Planck-Institute for Medical Research, Dept. of Biomedical Optics, Jahnstr. 29, D-69120 Heidelberg, Germany (E-mail: Rainer.Friedrich@mpimf-heidelberg.mpg.de).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
glomeruli (Baier and Korsching 1994; Byrd and Brunjes 1995). Moreover, several classes of natural odors have been identified for fish (Carr 1988). In the present study, we focused on amino acids, which are limited in number and activate a ventrolateral subregion of the OB that contains probably <200 MCs (Edwards and Michel 2002; Friedrich and Korsching 1997, 1998). Recently, we found that the slow temporal change of activity patterns across MCs during odor presentation results in a decorrelation of initially similar activity patterns, thereby enhancing their discriminability (Friedrich and Laurent 2001).

We compared odor responses from ORNs and MCs to examine the relationship between olfactory bulb input and output activity. Initially, odor responses of ORNs and MCs were similar in many respects. During the first few hundred milliseconds of odor presentation, however, multiple properties (variability, tuning, overall excitation) of MC responses, but not ORN responses, changed. These results provide insights into the processing of neural activity patterns in the OB.

METHODS

Preparation and odor stimulation

Adult zebrafish (Danio rerio) were obtained from a commercial supplier and kept under standard laboratory conditions at room temperature (23°C) for 2 wk before use. Electrophysiological experiments were performed in an explant preparation of the entire brain as described previously (Friedrich and Laurent 2001). Briefly, fish were anesthetized by cooling to 4°C and decapitated. The OBs and forebrain were exposed ventrally by removing the eyes, jaws, palate, and skull bones. The preparation was transferred upside-down into a custom-made flow chamber continuously perfused with teleost artificial cerebrospinal fluid (ACSF) and allowed to warm up to room temperature. Teleost ACSF contained (in mM) 124 NaCl, 2 KCl, 1.6 MgSO4, 2 CaCl2, 1.25 KH2PO4, 24 NaHCO3, and 10 glucose and was bubbled with 95% O2-5% CO2 (pH 7.25) (Mathieson and Maler 1988). All animal procedures were approved by the California Institute of Technology Animal Care and Use Committee with veterinary supervision by the Office of Animal Research.

A constant nasal flow of carrier medium was maintained throughout the experiment through Teflon tubing positioned in front of the ipsilateral nares. Odor stimuli (~2.4-s duration) were inserted into this carrier stream using an electronically triggered, pneumatically actuated injection valve (Valco). Amino acid solutions (Sigma, St. Louis, MO) were diluted from 1 mM stocks to a final concentration of 10 µM immediately prior to the experiment. Fresh stock solutions were made at least every 10 days.

Electrophysiology

Extracellular loose-patch recordings from MCs were performed using long-shank patch pipettes filled with ACSF (9–12 MΩ) (Friedrich and Laurent 2001). Signals were recorded with an Axoclamp 2B amplifier (Axon Instruments) in bridge mode and digitized at 10 kHz. Once a spike was detected extracellularly during a penetration, light suction was applied to establish a low-resistance seal. This procedure reliably isolated spikes from single neurons with good signal-to-noise ratio. In addition, the low seal resistance allowed simultaneous recording of the local field potential (LFP) from the same electrode. MCs were identified by their depth and the characteristic phase preference (~90°) of action potentials during periods of LFP oscillations. Spike times were extracted after off-line high-pass filtering at 280 Hz. LFPs were band-pass filtered off-line between 5 and 50 Hz using non-phase-shifting procedures. Most MC recordings were performed in the ventrolateral subregion of the OB that is activated by amino acids (Friedrich and Korsching 1997, 1998). All of the MCs within that region that were tested with the full panel of amino acids responded to at least one stimulus. MCs outside this region responded not at all or with weak inhibition to amino acid stimuli. A total of 272 MCs were recorded in 45 fish. In the amino acid-responsive region, responses to the full panel of 16 odors were collected from 58 MCs. Stimuli were repeated on average 3.2 ± 1 (SD) times.

Recordings from ORNs were performed using the same loose-patch technique. In most experiments, the skin over the rosette-like olfactory epithelium was removed to allow pipette access. The pipette was then inserted between the lamellae onto the central regions where ORNs reside. In some experiments, anterior lamellae were removed to facilitate pipette access.

Finding amino acid-responsive ORNs in the epithelium is difficult because they are intermingled with non-amino-acid-responsive types of ORNs. To facilitate selection, pilot experiments were performed in which ORNs were stimulated with each of the 16 amino acids as well as with a mixture of all 16 stimuli. Nine ORNs were found that did not respond to any of the single amino acids tested. These neurons did also not respond to the mixture. In eight ORNs that responded to at least one amino acid in pilot experiments, the mixture elicited a response that was similar to that of the most effective component amino acid, indicating that an ORN responsive to at least one of the amino acids also responds to their mixture. We therefore used the mixture stimulus to preselect amino-acid-responsive ORNs. Recordings were obtained from a total of 85 amino-acid-responsive ORNs in 14 fish. Twenty-three amino-acid-responsive ORNs were stimulated with the full panel of 16 amino acids [3.7 ± 1.4 (SD) repetitions of each odor]. The stability of ORN and MC responses was ensured in most experiments by reapplication of selected odors after stimulation with the standard odor set was completed.

Data analysis

The data set is an extension of that shown in a previous publication (Friedrich and Laurent 2001). All data analysis was carried out using routines written in Matlab (The MathWorks). Quantitative analysis was performed using the data from the 23 amino acid-responsive ORNs and 58 MCs that were stimulated with the complete set of 16 stimuli. For analysis of activity patterns, odor-evoked firing rates of single neurons were determined as the average firing rates measured in repeated applications of the same stimulus within a given analysis window. To analyze odor-evoked changes in firing rate (Fig. 3), the averaged pre-odor firing rates were subtracted. For the separate analysis of inhibitory and excitatory responses in histograms (Fig. 3, C and D), the sign of the firing rate change (positive or negative) was determined. Positive and negative firing rate changes were then summed separately.

VARIABILITY OF RESPONSE TIME COURSES. The variability of the response time course was assessed by comparing the shapes of peri-stimulus time histograms (PSTHs). Positive odor responses were selected from the data set if the firing rate of a neuron exceeded a threshold (30 Hz for ORNs, 40 Hz for MCs) in any time bin during odor presentation. The PSTHs were then normalized to the mean firing rate to obtain the shape independent of the absolute firing rate. The variance of normalized PSTHs was then calculated in 100-ms time bins, yielding a measure for the variability of the PSTH shape as a function of time.

TUNING WIDTH. The tuning width of ORN and MC response profiles was quantified by two independent measures, the sparseness and the half-width. The sparseness of a neuron across stimuli, it is called "lifetime" sparseness (Willmore and Tolhurst 2001). The sparseness S was calculated as $S = 1 - \frac{\text{threshold}}{\text{mean firing rate}}$.

This procedure reliably isolated spikes from single neurons with good signal-to-noise ratio. In addition, the low seal resistance allowed simultaneous recording of the local field potential (LFP) from the same electrode. MCs were identified by their depth and the characteristic phase preference (~90°) of action potentials during periods of LFP oscillations. Spike times were extracted after off-line high-pass filtering at 280 Hz. LFPs were band-pass filtered off-line between 5 and 50 Hz using non-phase-shifting procedures. Most MC recordings were performed in the ventrolateral subregion of the OB that is activated by amino acids (Friedrich and Korsching 1997, 1998). All of the MCs within that region that were tested with the full panel of amino acids responded to at least one stimulus. MCs outside this region responded not at all or with weak inhibition to amino acid stimuli. A total of 272 MCs were recorded in 45 fish. In the amino acid-responsive region, responses to the full panel of 16 odors were collected from 58 MCs. Stimuli were repeated on average 3.2 ± 1 (SD) times.

Recordings from ORNs were performed using the same loose-patch technique. In most experiments, the skin over the rosette-like olfactory epithelium was removed to allow pipette access. The pipette was then inserted between the lamellae onto the central regions where ORNs reside. In some experiments, anterior lamellae were removed to facilitate pipette access.

Finding amino acid-responsive ORNs in the epithelium is difficult because they are intermingled with non-amino-acid-responsive types of ORNs. To facilitate selection, pilot experiments were performed in which ORNs were stimulated with each of the 16 amino acids as well as with a mixture of all 16 stimuli. Nine ORNs were found that did not respond to any of the single amino acids tested. These neurons did also not respond to the mixture. In eight ORNs that responded to at least one amino acid in pilot experiments, the mixture elicited a response that was similar to that of the most effective component amino acid, indicating that an ORN responsive to at least one of the amino acids also responds to their mixture. We therefore used the mixture stimulus to preselect amino-acid-responsive ORNs. Recordings were obtained from a total of 85 amino-acid-responsive ORNs in 14 fish. Twenty-three amino-acid-responsive ORNs were stimulated with the full panel of 16 amino acids [3.7 ± 1.4 (SD) repetitions of each odor]. The stability of ORN and MC responses was ensured in most experiments by reapplication of selected odors after stimulation with the standard odor set was completed.

Data analysis

The data set is an extension of that shown in a previous publication (Friedrich and Laurent 2001). All data analysis was carried out using routines written in Matlab (The MathWorks). Quantitative analysis was performed using the data from the 23 amino acid-responsive ORNs and 58 MCs that were stimulated with the complete set of 16 stimuli. For analysis of activity patterns, odor-evoked firing rates of single neurons were determined as the average firing rates measured in repeated applications of the same stimulus within a given analysis window. To analyze odor-evoked changes in firing rate (Fig. 3), the averaged pre-odor firing rates were subtracted. For the separate analysis of inhibitory and excitatory responses in histograms (Fig. 3, C and D), the sign of the firing rate change (positive or negative) was determined. Positive and negative firing rate changes were then summed separately.

VARIABILITY OF RESPONSE TIME COURSES. The variability of the response time course was assessed by comparing the shapes of peri-stimulus time histograms (PSTHs). Positive odor responses were selected from the data set if the firing rate of a neuron exceeded a threshold (30 Hz for ORNs, 40 Hz for MCs) in any time bin during odor presentation. The PSTHs were then normalized to the mean firing rate to obtain the shape independent of the absolute firing rate. The variance of normalized PSTHs was then calculated in 100-ms time bins, yielding a measure for the variability of the PSTH shape as a function of time.

TUNING WIDTH. The tuning width of ORN and MC response profiles was quantified by two independent measures, the sparseness and the half-width. The sparseness S is a measure for the "peakedness" of a distribution (Rolls and Tovee 1995), normalized onto the interval between zero and one (Vinje and Gallant 2000). Applied to one neuron across stimuli, it is called "lifetime" sparseness (Willmore and Tolhurst 2001). The sparseness S was calculated as $S = 1 - \frac{\text{threshold}}{\text{mean firing rate}}$.
Thus activity patterns across MCs evolve during the early phase of the odor response and stabilize thereafter, whereas activity patterns across ORNs change little, consistent with previous results (Friedrich and Laurent 2001). The circuitry in the OB therefore transforms a constant input activity pattern into a time-varying output.

We next assessed the trial-to-trial variability in odor responses of ORNs and MCs by the CV of the odor-evoked firing rate in a sliding 100-ms time window (see Methods). The average CV of ORN responses decreased slightly at the beginning of the odor response and remained nearly constant thereafter, whereas the average CV of MC responses decreased further for ~1,000 ms before it stabilized (Fig. 2C). Thus MC firing rate responses are more reliable late during the odor response even though the average firing rate is lower (Friedrich and Laurent 2001).

Odors also evoked a 20- to 30-Hz oscillation in the LFP, reflecting synchronized activity of many neurons (Fig. 1B). This LFP was recorded simultaneously with single units from the same electrode. No LFP oscillation was observed in the olfactory epithelium (Dorries and Kauer 2000; Nikonov et al. 2002) at the odorant concentrations used here (not shown), indicating that the LFP oscillation was generated by local circuits in the OB.

Odor-evoked changes in activity across ORN and MC populations

We next analyzed single-unit responses evoked by the 16 stimuli across the population of ORNs and MCs. Figure 3A shows the changes in firing rates evoked by each of the 16 stimuli in 23 ORNs averaged over the duration of the stimulus. The color indicates positive (green-yellow) and negative (red) changes in firing rate, relative to the pre-odor firing rate (baseline). The average baseline firing rate of ORNs was 2.8 ± 2.2 (SD) Hz. With one exception (ORN 11 in Fig. 3A), ORNs responded with excitation to all effective odors. Odorants that did not excite ORNs did not detectably change the baseline firing rate. Weak inhibition, however, may have been difficult to detect against the low baseline firing rates. Of the 53 additional ORNs that were not stimulated with the full set of 16 amino acids, 2 responded exclusively with inhibition to all effective stimuli, whereas the other 51 ORNs did not show inhibition at all. These results suggest that ORNs may be classified as excitatory or inhibitory. Overall, 96% (73/76) ORNs were of the excitatory type and 4% (3/76) were of the inhibitory type. The net effect of odors on ORNs in zebrafish is therefore an increase in the population firing rate.

The average baseline firing rate of MCs was 8.8 ± 5.8 Hz. Because MC responses vary in time, activity patterns were analyzed using a sliding time window. Figure 3B compares odor-evoked MC activity in 400-ms time windows (centered on times indicated) immediately after response onset (left) and at later times. Like ORNs, MCs responded to multiple odorants. However, MCs were frequently inhibited by odors, and individual MCs usually responded with excitation to some odors and with inhibition to others. Ninety-eight percent (57/58) of MCs showed an inhibitory response component to at least one odor. Because inhibition is rare and static in ORNs, the inhibitory responses observed in MCs are likely caused by
synaptic interactions in the OB rather than by inhibition of ORNs by odors.

We next analyzed the overall changes in activity evoked by the 16 odorants in the recorded populations of ORNs and MCs. Figure 3, C and D, shows the summed firing rate changes relative to baseline evoked by each stimulus in the recorded ORNs and MCs, respectively. Excitatory and inhibitory responses are summed separately (I and H, respectively). This analysis again demonstrates that inhibition is rare in ORNs but frequent in MCs.

Individual amino acids evoked markedly different excitatory firing rate changes in the population of ORNs. At response onset, the odor-evoked total excitation in the MC population also depended on the odor. The relative potencies of the odors in exciting ORNs and MCs were similar (Fig. 3, C and D, left), suggesting that MC output initially follows its ORN input. Later during the response, however, odor-evoked excitatory MC population activity became more evenly distributed across odors. The total odor-evoked MC inhibition, in contrast, was evenly distributed across odors throughout the response. These trends were quantified by the CV of the firing rate changes across the different odorants as a function of time (Fig. 3E). The CV for excitatory MC responses (green) decreased during the first ~1 s of the odor response to ~40% of its initial value, whereas the CV for inhibitory responses (red) was lower and showed no clear trend. These data indicate that the reorganization of MC activity patterns is accompanied by a regulation of the overall excitation in the MC population toward a common level.

**Tuning width of ORNs and MCs**

MC response profiles (rows in matrices in Fig. 3B) changed over time, as shown previously (Friedrich and Laurent 2001). For example, MC 30 was initially excited by Arg and showed little response to Gly (Fig. 3B, left). Later, however, it was...
inhibited by Arg and excited by Gly (Fig. 3B, middle and right). We quantified the tuning width of MCs and ORNs, i.e., the response selectivity to the panel of amino acids, by the “lifetime” sparseness of the distribution of responses to the different odors (see Methods). Higher sparseness indicates a peakier distribution and, thus, more selective tuning. When averaged over neurons and time, responses of MCs and ORNs had similar lifetime sparseness (Fig. 4A). Similar results were obtained using the half-width of the response distribution as an alternative measure for response selectivity (Fig. 4B).

When analyzed over time (in successive 400-ms time windows advanced in 200-ms steps), the average tuning width did not change much for either ORNs or MCs (Fig. 4C) (Friedrich and Laurent 2001). The slight decline in the average sparseness of ORN responses probably results from the slowly decreasing firing rates of responding neurons relative to a constant background firing of nonresponding neurons (Fig. 1A) (Friedrich and Laurent 2001). However, individual neurons’ tuning widths behaved differently for ORNs and MCs. Figure 4, D and E, shows the lifetime sparseness of responses of each individual ORN and MC, respectively, as a function of time during odor presentation. In most ORNs, tuning widths did not change substantially over time except for the slight decline of sparseness observed also in the average tuning width (Fig. 4C). This is consistent with the low variance of ORN responses over time (Fig. 2A). The tuning of individual MCs, by contrast, changed profoundly during odor stimulation (Fig. 4E) and could undergo sharpening (e.g., Fig. 4E, MC 26), broadening (e.g., Fig. 4E, MC 34), or, in rare cases, more complex changes (e.g., Fig. 4E, MC 37).

The time courses of tuning width were further analyzed by the Pearson correlation coefficient between the change of sparseness over time for individual ORNs and MCs (Fig. 4, D and E, respectively) and the corresponding average curves (Fig. 4C). This measure does not depend on the overall sparseness but exclusively on the change of sparseness over time (i.e., the shape of the curves in Fig. 4, C–E). The average correlation coefficients were high (Fig. 4F; mean ± SD: 0.79 ± 0.23). Ninety-one percent (21/23) of ORNs showed a statistically significant (P < 0.05) correlation between the time course of lifetime sparseness and the average time course. Hence, most or all ORNs show a similar change of response sparseness over time, namely, a slight decline. For MCs, correlations between the time course of lifetime sparseness and the average time course were significantly lower (Fig. 4G; 0.29 ± 0.34; P < 10^-7; Wilcoxon rank-sum test) and statistically significant for only 21% (12/58) of MCs. The time courses of tuning width across MCs are therefore significantly more diverse than those of ORNs. The direction of change in tuning width during odor presentation was assessed by the correlation between sparseness and time for individual ORNs and MCs. For 87% (20/23) of ORNs, sparseness and time were significantly correlated (P < 0.05). In all cases, the correlation coefficient was negative, reflecting the slight decline of sparseness over time seen in most ORNs and in the average curve (Fig. 4C). Among MCs, 69% (40/58) showed a significant correlation between sparseness and time. In 40% of these MCs (16/40), the correlation coefficient was negative, indicating that sparseness decreased over time, whereas in the remaining 60%, the correlation coefficient was positive and sparseness increased over time. Hence, the response specificity changes significantly over time in a substantial fraction of MCs. Specificity of individual MCs can change in both directions, while the average tuning width in the population remains approximately constant.

**Odor identification from activity patterns**

The change of MC firing during the first few hundred milliseconds of the odor response results in a decorrelation of initially similar activity patterns evoked by related odors.
FIG. 3. Response profiles and population responses of ORNs and MCs. A: color-coded matrix showing firing rate changes, relative to the pre-odor firing rate, evoked by 16 odors (10 μM; x axis) in 23 ORNs (y axis; arbitrary order). Because ORN responses follow a stereotyped time course, firing rates were averaged over the stimulus duration. Green/yellow colors indicate an odor-evoked increase in firing rate, red colors indicate a decrease. Firing rate changes were normalized to the largest absolute firing rate change for each neuron. ORNs responded to multiple odorants. All but 1 ORN (ORN 11) responded with excitation to all effective odorants. B: color-coded matrix of firing rate changes evoked by the same odorants in 58 MCs. Because response time courses of MCs are complex, firing rates were measured within 400-ms windows centered on 3 different time points indicated above. Like ORNs, MCs are excited by multiple odorants; unlike in ORNs, inhibition is observed frequently. A given MC can be excited by some odorants and inhibited by others. Over time, response profiles of individual MCs change. C: sum of firing rate changes evoked by each odor over all ORNs. Excitatory (■) and inhibitory firing rate changes (□) are depicted separately. The total stimulus-evoked change in firing rate differs substantially across odorants, and inhibition is rare. D: sum of excitatory and inhibitory firing rate changes in the population of MCs measured within 400-ms windows centered at times indicated above. A response was classified as excitatory or inhibitory when the firing rate was higher or lower, respectively, than the baseline firing rate. Inhibition is prominent throughout the odor response. Initially, the profile of total excitatory firing rate changes across odorants was similar to that for ORNs. Later, the distribution of firing rate changes became more even. E: the variation of firing rate changes evoked by different odors in MCs, quantified by the CV, as a function of time.
Analysis of tuning width in ORNs and MCs. A: comparison of average ORN and MC tuning width assessed by the sparseness of response profiles (see METHODS). Sparseness of response profiles was determined for each neuron in a 400-ms time window every 200 ms and averaged over time and neurons. The slightly higher sparseness (sharper tuning) of MC response profiles was not statistically significant ($P = 0.13$, Wilcoxon rank-sum test). B: comparison of average ORN and MC tuning width assessed by the half-width of tuning (see METHODS). Analogous to sparseness, half-width was determined in 400-ms windows every 200 ms and averaged over time and neurons. The slightly higher half-width (broader tuning) found for MCs was weakly significant ($P = 0.024$, Wilcoxon rank-sum test). C: average tuning width (mean ± SD) of ORNs (gray) and MCs (black) as a function of time, assessed by sparseness. E: tuning width (sparseness) of all 23 ORNs as a function of time. The tuning of individual ORNs did not change profoundly over time (except for a slight decline in sparseness observed also in the average; see C). F: tuning width (sparseness) of all 58 MCs as a function of time. The tuning of individual MCs could broaden (e.g., MC 34), sharpen (e.g., MC 26) or undergo more complex changes (e.g., MC 37) during odor presentation. G: distribution of correlation coefficients between the time courses of individual ORNs’ response sparseness (E) and the time course of the average response sparseness of ORNs (C). Most correlation coefficients are high. H: distribution of correlation coefficients between time courses of individual MCs’ response sparseness (F) and their average (C). A substantial proportion of correlation coefficients are low or negative, indicating that the time courses of tuning width differ considerably across the population of MC.
(Friedrich and Laurent 2001). Concomitantly, odor identification by a pattern matching algorithm from MC firing patterns in short time windows improves substantially. This improvement may result from the decorrelation of activity patterns over time or from the decreasing variability of spiking during the odor response (Fig. 2C). To distinguish between these possibilities, we tested the dependence of odor identification on the number of MCs in the ensemble pattern. If the improvement of odor identification is due to the decorrelation of activity patterns, it should gracefully degrade with decreasing numbers of MCs in the pattern because the effect is observed only at the population level. If the improvement of odor identification is due to a decrease in response variability over time, it would be expected to be less sensitive to the number of neurons in the ensemble. Furthermore, we wished to determine whether odor identification based on activity patterns across ORNs also improves over time.

Odor identification was performed by a template matching algorithm based on the measured spike trains. The procedure is explained in detail in Fig. 5A. It matches a pattern of firing rates, constructed from single-trial responses of s neurons to a randomly selected test odor, to template activity patterns, constructed in the same manner from other single-trial responses of the same s neurons to all 16 odors. If the best match is between the test pattern and the template for the same odor, identification is correct; otherwise an error is counted. The procedure is then repeated ≥1,000 times, each time drawing a new set of s neurons, a new test odor, and new single trials from the data set. The error rate as a function of time was then determined for MC ensembles of different size by varying s. Patterns of firing rates across neurons were determined in an analysis window, usually 400 ms long, that was stepped over the odor presentation period in 100-ms increments. This relatively long time window was chosen to explore the effect of slow temporal response dynamics independently of the faster rhythmic activity occurring on the 35- to 50-ms time scale.

Odor identification based on responses from single MCs (s = 1) was better than chance level (1 – 1/16 = 93.75% errors) but improved not or only slightly over the course of the odor response (Fig. 5B; 1 MC), indicating that the decrease in response variability of single neurons does not affect odor identification from single MCs’ responses. Increasing the number of MCs in the pattern decreased overall error rates (Fig. 5, B and C). In addition, odor identification also improved over time. The error rate was always highest at response onset and decreased during the subsequent ~1,000 ms. The relative improvement in odor identification over time was quantified by the ratio of the error rate at response onset (1st 400-ms time window) to that at the end of odor presentation (average over last 10 error rate values, each from 1 400-ms time window). The improvement in odor identification over time increased with increasing pattern size from 1.1-fold for s = 1 to 10.9-fold for s = 55 (Fig. 5D). This effect was also apparent from the dependence of odor identification on pattern size when analyzed separately for early and late times of the odor response: at both times, odor identification improved with pattern size, but the rate of improvement was faster for late response times (Fig. 5C; steeper slope for late patterns). Hence, the relational information in activity patterns, but not the responses of the same MCs when considered independently, became progressively more informative over time, indicating that the reorganization of activity patterns significantly improved odor identification.

Analysis windows of different length (100–800 ms) changed the overall error rate, with longer time windows giving lower error rates. This is expected because more spikes contribute to each pattern. The relatively low error rates obtained with 55 MCs in Fig. 5B therefore depend on the relatively long analysis window. For all time windows tested, however, the same qualitative dependence on pattern size and response time was observed: with increasing pattern size, the overall error rate decreased and the improvement of odor identification over time became greater. Hence, the improvement of pattern-based odor identification over time, illustrated for a 400-ms analysis window in Fig. 5B, does not critically depend on the analysis window length.

Odor identification based on activity patterns across the 23 sampled ORNs followed a different time course. The error rate initially decreased slightly but subsequently increased (Fig. 6; gray curve). Odor identification based on the same number of MCs (s = 23), in contrast, decreased and remained low throughout odor presentation (Fig. 6; black curve). The time course of the error rate of ORN-based odor identification may reflect the overall firing rate, which increases fast after stimulus onset and then decays slowly (Friedrich and Laurent 2001). Thus as the firing rate of responding ORNs decreases while the basal firing rate of nonresponding ORNs remains constant, the contrast in odor-evoked activity patterns deteriorates, making odor identification more difficult. These results show that activity patterns across MCs become more informative about the identity of a stimulus over time, although their inputs do not, suggesting that the OB accumulates odor information in the dynamic reorganization of activity patterns.

**DISCUSSION**

We dynamically analyzed the responses of inputs (ORNs) and outputs (MCs) of the zebrafish OB to a well-defined set of 16 natural amino acid stimuli. Several attributes of MC, but not ORN, activity changed during the first ~1,000 ms of an odor response: MC response variability decreased, the tuning of individual MCs changed, overall excitation converged to an odor-independent common level, and patterns of activity became more informative about odor identity. In addition, MC firing evolved from intense but asynchronous to weaker but rhythmically synchronized activity across MCs (Friedrich and Laurent 2001). The absence of these effects in ORNs indicates that the dynamic reorganization of MC activity results from synaptic interactions in the OB and that the dynamic redistribution of inhibition plays an important role in this process.

**Response properties of individual ORNs and MCs**

Most ORN responses were excitatory and followed a stereotyped, phasic-tonic time course. Inhibitory responses of ORNs were rarely observed. This may be partially due to the low baseline firing rate, which complicates the detection of weak inhibitory responses. However, strong excitation and inhibition were never observed in the same ORN. ORNs may therefore fall into two functional classes (excitatory or inhibitory). Because of the preponderance of excitatory responses, an odor stimulus elicits a net firing rate increase across inputs to the
OB. This is consistent with odor-evoked changes in calcium concentrations in glomerular afferents of the zebrafish (Friedrich and Korsching 1997; Fuss and Korsching 2001) and odor responses of mammalian ORNs (Duchamp-Viret et al. 1999; Getchell 1986), but unlike ORN responses of catfish ORNs (Kang and Caprio 1995).

MC odor responses were often multiphasic, and inhibition was frequently observed. Unlike responses of ORNs, inhibition in MCs was often transient and alternated with excitatory epochs in the same response. These properties of inhibition in MCs indicate that it comes predominantly from interneurons in the OB rather than from the silencing of ORN input. The OB output conveyed by a MC is therefore not strictly determined by its sensory input but significantly influenced by network interactions within the OB.

The trial-to-trial variability of individual MCs’ responses decreased markedly over time, whereas the variability of ORN responses decreased only slightly. At the same time, the aver-

FIG. 5. Odor identification by odor-evoked activity patterns. A: schematic illustration of the template matching algorithm. One odor was randomly selected as the test odor. From each MC, a single response to the test odor was taken and the firing rate measured within the analysis window, resulting in a vector representation of the firing rate pattern (test vector). In the same manner, templates were constructed from single trial responses of the same MCs for all 16 odors. Trials selected for the test vector were excluded from templates. The test vector was assigned to the stimulus to whose template it was most highly correlated. If the “identified” odor was not the original test odor, an error was counted. The error rate was determined from ≥1,000 iterations of the procedure at each time point, each time drawing a random test odor, a random set of MCs, and random single responses. The time dependence of the error rate was assessed by stepping the analysis window (usually 400 ms long) forward in time in 100-ms increments. B: error rate of odor identification as a function of time and number of MCs. With increasing numbers of MCs, error rate decreased overall. In addition, odor identification improved over time, and this effect became more pronounced with increasing numbers of MCs. C: error rate as a function of number of MCs. Dashed line, error rate averaged over all 400-ms time windows. Black line, error rate at response onset (0- to 400-ms window; onset). Gray line, error rate during the last second of the response (average over error rate values from the last 10 time windows; late; analysis windows were always 400 ms long). Error rate decreased with pattern size, but the decrease was steeper for “late” than “onset” activity patterns. D: quantification of improvement of odor identification during odor presentation, measured as the ratio of error rates for onset and late activity patterns. The relative improvement in odor identification over time increased with the number of MCs in the pattern.
Average firing rates of ORNs and MCs first increase and then decrease (Friedrich and Laurent 2001). Hence, late MC response phases are more reliable than early ones although the average firing rate is lower, implying that more information is conveyed per spike. In projection neurons of the locust antennal lobe, the response reliability increases over repeated applications of the same odor (Stopfer and Laurent 1999). It is, however, unclear how these two forms of unsupervised plasticity are related because one occurs within a stimulus (over hundreds of milliseconds), whereas the other occurs over repeated stimuli (over seconds to minutes).

Tuning profiles of individual MCs changed substantially during odor presentation (see also Friedrich and Laurent 2001). Tuning profiles are not merely modulated in width, but they change in shape. It is therefore not appropriate to describe MC odor tuning by a single “tuning curve.” Individual MCs’ tuning could sharpen or broaden during odor presentation; on average, however, tuning width remained almost constant. The tuning of a MC is determined by its input from ORNs and by inhibitory interactions in the OB. The pattern of ORN activity is mostly excitatory and relatively stable during the odor response (except for a slow adaptation of firing rates). The dynamic properties of MC responses indicate that inhibitory response phases are caused predominantly by synaptic input from interneurons in the OB. Inhibitory response epochs in MCs are often transient and contribute much to the change of MC tuning over time. Hence, the dynamics of MC response profiles appear to result, at least in part, from a redistribution of inhibition in the OB during an odor response.

The synaptic mechanisms underlying the response dynamics of MCs remain to be explored. Experiments in other species have shown that slow temporal patterning of MC activity persists after severing connections of the OB to and from other brain structures. Feedback from higher brain regions therefore does not appear to be required for pattern dynamics, although it may play a modulatory role (Meredith and Moulton 1978; Schild et al. 1987). In the antennal lobe of insects, adaptation of inhibitory neurons and slow synaptic inhibition appear to be involved in generating slow temporal dynamics of the activity of the MC analogues, the projection neurons (Bazhenov et al. 2001a,b; MacLeod and Laurent 1996).

Input-output relationship of activity patterns in the OB

At response onset, OB output activity patterns appear to be closely related to their inputs as indicated by two observations. First, the relative effectiveness of the odors in eliciting excitatory responses was similar for ORNs and MCs, suggesting that MCs are driven by their sensory inputs. Second, odors that evoked similar activity patterns across ORNs also evoked similar patterns of MC activity (Friedrich and Laurent 2001). As the odor response progressed, however, MC activity patterns were reorganized and the similarity relationships changed. In addition, the total MC excitation evoked by different odors approached a common level (Fig. 3, D and E). Hence, both the structure and the overall intensity of activity patterns are reorganized during the first few hundred milliseconds of the odor response.

The convergence of the total excitatory firing rate change across MCs evoked by different odors may serve to keep the total activity within a range appropriate for proper network function. This may be important because the density and intensity of sensory input evoked by natural odors varies dramatically (Friedrich and Korsching 1997). The mechanisms underlying the regulation of excitation in the OB are not understood but may include known connections such as recurrent and lateral inhibitory synaptic interactions between MCs and granule cells (Aroniadou-Anderjaska et al. 1999, 2000; Chen et al. 2000; Isaacsen and Strowbridge 1999; Margrie et al. 2001; Schoppa and Westbrook 2001).

It has been proposed (Mori et al. 1999; Yokoi et al. 1995) that local inhibitory connections between MCs receiving similarly tuned input systematically sharpen MC response profiles in a manner akin to receptive field sharpening in the visual and auditory systems (Hartline and Ratliff 1957; Yang et al. 1992). However, response profiles of MCs are not static but change over time presumably due to a redistribution of inhibition within the OB. This is not consistent with the classical model of contrast enhancement by lateral inhibition derived from other sensory systems. We therefore propose that an important role of the neural circuitry in the OB is to promote dynamic computations at the level of population activity, such as the decorrelation of similar patterns of input activity (Friedrich and Laurent 2001).

Odor identification from dynamic activity patterns

The discriminability of odor-evoked activity patterns was explored using a pattern matching algorithm, which has obvious limitations. In particular, the role of fine temporal correlations (on a time scale less than ~100 ms) could not be analyzed because it would require simultaneous recordings from a large number of neurons. An advantage of the algorithm is that it does not require prior knowledge about spike train statistics.

The reliability of odor identification based on patterns of ORN activity decreased during later response phases. Overall, the time course of the error rate was similar to the inverse of the firing rate of ORN odor responses (Friedrich and Laurent 2001), suggesting that the error rate may be determined mostly by the change in contrast of the odor-evoked firing pattern as sensory responses adapt.

The reliability of MC-based odor identification, in contrast,
followed a time course different from that of ORN-based odor identification or the average time course of MC firing rates (Friedrich and Laurent 2001) and was therefore analyzed in more detail. The reliability of odor identification from single MCs’ responses remained almost unchanged during the odor response, indicating that the decreasing response variability has little impact on odor identification by the algorithm used here. However, it is possible that increasing reliability becomes important for the readout of information contained in the fine temporal structure of activity patterns (Perez-Orive et al. 2002; Stopfer and Laurent 1999; Wehr and Laurent 1996).

Odor identification based on activity patterns, in contrast, improved substantially, with a time course similar to that of pattern decorrelation (Friedrich and Laurent 2001). Decreasing the number of MCs in a pattern resulted in a graceful degradation of this effect, indicating that odor-evoked activity patterns become more informative about odor identity, most likely because of the decorrelation of activity patterns evoked by similar odors (Friedrich and Laurent 2001). These results imply that activity patterns become more informative not because single MCs’ responses become more specific but because activity patterns are reorganized so that information in relational features of activity patterns is enhanced. Hence, sustained sensory input is not simply integrated over time, but information is accumulated in a stimulus-specific sequence of activity patterns that become progressively more informative.

Conclusions

Our analysis of odor responses at the input and output level of the OB demonstrated that synaptic interactions in the OB dynamically alter multiple aspects of neuronal activity in the early olfactory pathway. At all times and synaptic stages examined, however, odor information is contained in combinatorial patterns of activity across multiple neurons with little change in the average odor selectivity of individual neurons. Hence, neural circuits in the OB change the format but not the combinatorial nature of odor representations. Consequences of processing in the OB are an enhanced discriminability of odor-encoding MC activity patterns and a regulation of the overall excitation. It appears likely that further consequences remain to be discovered.

Acknowledgments

We thank A. Schäfer, H. Spors, and the Friedrich lab for discussions and comments on the manuscript.

Grants

This work was supported by the National Institute of Neurological Disorders and Stroke, Keck Foundation, McKnight Foundation, and Max-Planck-Society.

References


