Dynamics of olfactory bulb input and output activity during odor stimulation in zebrafish

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The processing of odor-evoked activity in the olfactory bulb (OB) of zebrafish was studied by extracellular 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), while ORN response profiles remained nearly unchanged. Several observations indicate that dynamic inhibition plays an important role in this remodelling. Finally, the reliability of odor identification based on MC population activity patterns improved over time, while 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.

Key Words: Olfactory bulb, odor coding, neural dynamics, activity pattern, zebrafish
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 odorants, and a given odorant activates multiple glomeruli. Hence, odor identity is represented combinatorially by patterns of glomerular activation. Studies using optical imaging and other techniques in the OB and its insect equivalent, the antennal lobe (AL), have explored the organization of glomerular activity patterns and their relationships to chemical properties of odorants (Cinelli et al. 1995; Fried et al. 2002; Friedrich and Korsching 1997; Fuss and Korsching 2001; Johnson and Leon 2000; Meister and Bonhoeffer 2001; Rubin and Katz 1999; Sachse et al. 1999; Uchida et al. 2000; Wachowiak and Cohen 2001). These studies, together with earlier investigations, revealed that chemically related odorants evoke similar afferent activity patterns, presumably because they bind to overlapping sets of odorant receptors (Bozza et al. 2002; Malnic et al. 1999; Zhao et al. 1998).

Input activity is transmitted to the principal neurons of the OB, the mitral cells (MCs; in higher vertebrates also tufted cells), which convey the output of the OB. MCs make various synaptic and asynaptic connections in the OB, including reciprocal dendro-dendritic synaptic connections with local GABAergic interneurons, the granule cells (Isaacson and Strowbridge 1999; Shepherd and Greer 1998; Shipley and Ennis 1996). Similar interactions exist in the AL between principal neurons, the projection neurons (PNs), and local inhibitory interneurons (Laurent 1996). These interactions shape MC and PN odor response profiles by recurrent and lateral

The spike output generated by a MC is determined both by its sensory input and by interactions with interneurons in the OB. Synaptic influences from interneurons depend on the activity pattern in the OB, which in turn depends on the glomerular activity pattern evoked by an odor stimulus. Therefore, the relationship between sensory input and spike output of a MC may not be constant, but modulated by network interactions in a complex and odor-dependent manner. As a result, the odor response profile of a MC may differ from that of its glomerular input. Odor information is, most likely, encoded by the activity of multiple neurons in the early olfactory pathway. It is therefore of particular importance to understand the conversion of patterns of sensory input into patterns of MC output.

We started to address these questions in the OB of zebrafish, which is an attractive model system because it is similar to that of larger vertebrates but contains relatively few neurons and 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 focussed on amino acids, which are limited in number
and activate a ventro-lateral subregion of the OB that contains probably less than 200 MCs (Edwards and Michel 2002; Friedrich and Korsching 1998, 1997). 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 at least two weeks before use. Electrophysiological experiments were performed in an explant preparation of the entire brain as described previously (Friedrich and Laurent 2001). Briefly, fish were anaesthetized 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 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, 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 naris. 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 deg.) of action potentials during periods of LFP oscillations. Spike times were extracted after off-line high-pass filtering at 280 Hz.
LFPs were bandpass filtered offline between 5 and 50 Hz using non-phase shifting procedures. Most MC recordings were performed in the ventro-lateral subregion of the OB that is activated by amino acids (Friedrich and Korsching 1998, 1997). 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 odorants were collected from 58 MCs. Stimuli were repeated on average 3.2 ± 1.1 times (mean ± SD).

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 alone, 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 pre-select 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 repetitions of each odor; mean ± SD).

The stability of ORN and MC responses was ensured in most experiments by reapplication of selected odorants 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. 3C, 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 “peakiness” 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

$$S = \left\{1 - \frac{\left(\sum r_n/N\right)^2}{\sum (r_n^2/N)}\right\} / \left[1 - (1/N)\right]$$

(Vinje and Gallant 2000), where $r_n$ is the response to odor $n$ and $N = 16$ is the total number of odors. In this notation, low values indicate broad tuning and high values indicate narrow tuning. The half-width is a measure for the width of a distribution. It is more intuitive than sparseness, but discards information about the shape of the distribution. Half-width was determined for each response profile by ranking the 16 odor responses in decreasing order and finding the rank at which the firing rate was equal to half the maximum rate over all 16 odors. Fractions of ranks were found by interpolation. Contrary to sparseness, low values indicate narrow tuning and high values indicate broad tuning.

**Trial-to-trial variability of firing rates.** Variability of odor-evoked firing rate within a given analysis window was quantified by the average coefficient of variation (CV). The CV (standard deviation normalized to the mean) was determined within a given analysis window for a given neuron and odor from the firing rates evoked by repeated applications (trials) of that odor. CVs were then averaged over all neurons (ORNs or MCs) and odors. In some cases, the average firing rate within a given analysis window for a given odor was zero; the CV is then not defined. In this case the CV was set to zero. Setting the CV to one produced similar results. The CV, rather
than the standard deviation, was used as a measure for response variability because it is independent of the overall firing rate in the population, which varied over time (Friedrich and Laurent 2001). A decrease in the standard deviation could thus simply reflect a decrease in firing rate, with the relative variability remaining constant. A decrease in the CV over time indicates, however, that the variability decreases even relative to the average firing rate.

RESULTS

Responses to the complete panel of 16 amino acid odorants (10 µM) were recorded from 23 amino acid-responsive ORNs and 58 MCs. Responses to a subset of the 16 odorants were obtained from an additional 53 ORNs and 214 MCs.

Basic properties of ORN and MC responses

Individual ORNs and MCs usually responded to multiple odorants. As described previously (Friedrich and Laurent 2001), ORN responses followed a stereotyped phasic-tonic time course, while MCs responses displayed complex and odor-specific temporal patterns, often consisting of successive excitatory and inhibitory phases (Fig. 1A, B). ORN and MC responses showed no obvious changes throughout the course of the experiment, as assessed by re-application of odorants after intervening stimulation with other odorants (Fig. 1C, D). The diversity of response time courses evoked by different stimuli was quantified by the variance of normalized PSTHs in 100 ms time bins (see Methods).
The variance of temporal response patterns was much higher for MC than for ORNs, especially during the first second of the response (Fig. 2A). These data imply that odor-evoked activity patterns across ORNs are phasic-tonically modulated in intensity, but do not change much during odor presentation. Activity patterns across MCs, in contrast, change over time, as some MCs cease firing while others start firing. The rate of change of activity patterns was quantified as the average correlation between subsequent firing patterns, averaged in 200 ms bins, across all recorded ORNs and MCs. The correlation between successive ORN activity patterns was > 0.9 throughout the odor response, whereas successive MC activity patterns were correlated more weakly during the first ~1000 ms of the response (Fig. 2B). Thus, activity patterns across MCs evolve during the early phase of the odor response and stabilize thereafter, while 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 coefficient of variation (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 ~1000 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 – 30 Hz oscillation in the local field potential (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. Fig. 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 Hz (mean ± SD). With one exception (ORN #11 in Fig. 3A), ORNs responded with excitation to all effective odorants. 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. Out 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, while 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 (mean ± SD). Because MC responses vary in time, activity patterns were analyzed using a sliding time window. Fig. 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. Since inhibition is rare and static in ORNs, the inhibitory responses observed in MCs is 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. Figs. 3C and D show 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 (filled and open bars, 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. 3C 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 odorants. 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 about 40% of its initial value, while 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 towards 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 non-responding neurons (Fig. 1A) (Friedrich and Laurent 2001). However, *individual* neurons’ tuning widths behaved differently for ORNs and MCs. Figs. 4D and E show 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 (Figs. 4D 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 Figs. 4C - E). For ORNs, 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; mean ± SD: 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, while 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 (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 at least 1000 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 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 35-50 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. 5B, C). In addition, odor identification also improved over time. The error rate was always highest at response onset and decreased during the subsequent \(~1000\) ms. The relative improvement in odor identification over time was quantified by the ratio of the error rate at response onset (first 400 ms time window) to that at the end of odor presentation (average over last 10 error rate values, each from one 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 non-responding 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 in zebrafish. Several attributes of MC, but not ORN, activity changed during the first ~1000 ms of an odor response: (1) MC response variability decreased, (2) the tuning of individual MCs changed, (3) overall excitation converged to an odor-independent common level, and (4) 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-tomic 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, while the variability of ORN responses decreased only slightly. At the same time, the 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, since one occurs within a stimulus (over hundreds of ms), while 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 does therefore 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; Bazhenov et al. 2001b; 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. 3D, E). Hence, both the structure and the overall intensity of activity patterns are reorganized during the first few hundred ms 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; Aroniadou-Anderjaska et al. 2000; Chen et al. 2000; Isaacson 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 < ~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 due to 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.

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REFERENCES


FIGURE LEGENDS

FIG. 1. Odor responses of ORNs and MCs. A: responses of an ORN to five amino acid stimuli (10 µM). Rasters depict action potentials in repeated trials, peri-stimulus time histograms (PSTHs) show average firing rates in 100 ms bins. Odor stimulus is indicated by the gray shading. Note that the ORN responds to a subset of stimuli with different intensities, but with a stereotyped phasic-tonic time course. B: responses of a MC to five amino acids (10 µM). Responses were chosen to illustrate typical range of response types. Note the odor-specific response time courses including excitatory and inhibitory epochs. A recording of the LFP (5 – 50 Hz band) from the same electrode is shown above each panel. C, Responses of the same ORN to two blocks of stimulation with the same odorant (Trp, 10 µM), separated in time by 50 min. In the intervening time, other odor stimuli were applied. D, Responses of the same MC to two blocks of stimulation with the same odorant (Lys, 10 µM), separated in time by 49 min. Note stability of odor responses of ORN (C) and MC (D).

FIG. 2. Properties of ORN and MC responses as a function of time. A: time-dependent variability of response time courses, measured as the variance of normalized PSTHs in each 100 ms time bin. B: Rate of change of activity patterns as a function of time. The rate of change was assessed as the correlation between an activity pattern at time t and the previous pattern. A low correlation indicates a large change within a 200 ms time step. While ORN activity patterns change little, MC activity patterns change more rapidly during the first second and stabilize thereafter. C: The trial-to-trial variability of firing rates in 400 ms time bins was measured from repeated odor applications as the coefficient of variation (CV) for each neuron and odor, and then
averaged over neurons and odors (see Methods). Because the CV is a normalized measure of variability, this measure is independent of changes in total firing rate over time. Error bars show SD.

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 odorants (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 one 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 three 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 (filled bars) and inhibitory firing rate changes (open bars) 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, quantitified by the CV, as a function of time.

FIG. 4. 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 (\( \pm \) 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.

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 a 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 at least 1000 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, s. 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 indicates error rate averaged over all 400 ms time windows. Black line indicates error rate at response onset (0 – 400 ms window; onset). Gray line indicates 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.

FIG. 6. Comparison of odor identification based on ORN and MC activity patterns. The error rate as a function of time was determined based on activity patterns of 23 ORNs and 23 MCs. While odor identification by ORN patterns degrades, identification by MCs patterns improves over time.
Figures

A. Variance vs. Time (s)

B. Correlation between successive patterns vs. Time (s)

C. Average CV vs. Time (s)
A. Bar graphs showing the average sparseness of ORNs and MCs. The y-axis represents sparseness, and the x-axis represents the type of neuron (ORNs or MCs). The error bars indicate the standard deviation.

B. Bar graphs showing the average half-width of ORNs and MCs. The y-axis represents half-width, and the x-axis represents the type of neuron (ORNs or MCs). The error bars indicate the standard deviation.

C. Line graph showing the correlation coefficient over time for ORNs and MCs. The x-axis represents time in seconds, and the y-axis represents the correlation coefficient.

D. Graphs of sparseness over time for receptor neurons. Each panel shows a different neuron, identified by a number.

E. Graphs of sparseness over time for mitral cells. Each panel shows a different neuron, identified by a number.

F. Histogram showing the distribution of correlation coefficients for receptor neurons. The x-axis represents the correlation coefficient, and the y-axis represents the number of neurons.

G. Histogram showing the distribution of correlation coefficients for mitral cells. The x-axis represents the correlation coefficient, and the y-axis represents the number of neurons.
Randomly select odor
Randomly select single trials

Firing rate
Test odor vector

Match against templates for all odors
Highest correlation: “identified” odor

A

B

C

D

Friedrich & Laurent, Fig. 5
Friedrich & Laurent, Fig. 6