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History-dependent odor processing in the mouse olfactory bulb

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Abbreviated title: Odor coding in face of background

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Abstract

In nature, animals normally perceive sensory information on top of backgrounds. Thus, the neural substrate to perceive under background conditions is inherent in all sensory systems. Where and how sensory systems process backgrounds is not fully understood. In olfaction, only few studies addressed the issue of odor coding on top of continuous odorous backgrounds. Here, we tested how background odors are encoded by mitral cells (MCs) in the olfactory bulb (OB) of male mice. Using *in vivo* two-photon calcium imaging we studied how MCs responded to odors in isolation versus their responses to the same odors on top of continuous backgrounds. We show that MCs adapt to continuous odor presentation and that mixture responses are different when preceded by background. In a subset of odor combinations, this history dependent processing was useful to promote identification of target odors over background. Other odorous backgrounds were highly dominant, such that target odors were completely masked by their presence. Our data are consistent in both low and high odor concentrations and in anesthetized and awake mice. Thus, odor processing in the OB is strongly influenced by the recent history of activity, which could have strong impact on how odors are perceived.

Significant statement: We examined a basic feature of sensory processing in the olfactory bulb. Specifically, we measured how mitral cells adapt to continuous background odors and how target odors are encoded on top of such background. Our results show clear differences in odor coding based on the immediate history of the stimulus. Our results support the argument that odor coding depends on the recent history of the sensory environment, already at the level of olfactory bulb output.
Introduction

Natural environments are comprised of complex stimuli, which are actively processed by the nervous system to extract behaviorally relevant information. Moreover, at any given time point, animals face sensory environments that were immediately preceded by other environments. The short history preceding a sensory stimulus may contain vital information for the animal or simply be noise that should be suppressed. Behaviorally, animals seamlessly deal with background and are able to perceive details in noisy and dynamic scenes. In other cases, background can be so dominant that it precludes the perception of novel odors.

Backgrounds have been shown to alter processing in various sensory systems. For example, in the retina, neurons increase their sensitivity in response to visual patterns that are differentially oriented from their background (Hosoya et al., 2005). In the visual cortex of cats, adaptation was shown to be pattern selective (Movshon and Lennie, 1979). In the primary auditory cortex of mice, responses are highly sensitive to the background with which they are embedded in time and frequency (Williamson et al. 2016). Similarly, in the barrel cortex of rats, high sensitivity across whiskers is maintained following an adaptive response to high frequency whisker stimulation (Katz et al., 2006). In olfaction, neural correlates of background processing have been studied only in few cases, so our understanding of these processes remain rudimentary.

Olfactory backgrounds are often continuous and dynamic. As such, they must be processed on both fast and slow time scales. Although significant computations do occur at short time scales, the effects of slowly changing or continuous stimuli on odor coding are normally not addressed (Cleland, 2010; Koulakov and Rinberg, 2011; Carandini and Heeger, 2012; Friedrich and Wiechert, 2014). Moreover, empirical data on the extent to which slow odor responses change and how these contribute to coding are not only scarce but conflicting. In some studies, background odor information passing through the OB was reported to remain unperturbed (Kadohisa and Wilson, 2006; Stevenson and Wilson, 2007; Gottfried, 2010; Tong et al., 2014), while in other studies it was shown to be dynamically changing (Bathellier et al., 2008; Adam and Mizrahi, 2011; Patterson et al., 2013). Prevailing ideas suggest that neural correlates of odor coding over background in
the mammalian olfactory system start to appear only in the piriform cortex where strong adaptation to constant background odors has been measured (Gottfried, 2010; Wilson and Sullivan, 2011).

Here we studied odor coding in face of continuous background in the mouse OB using two-photon calcium imaging. We asked how MCs process background odors and, in turn, how background odors affect transient odor evoked MCs responses. Our results suggest that odor coding is history dependent because odor responses to the same mixture stimulus changes when preceded by background. Moreover, history-dependent coding in the OB can either promote an effective detection of target odors or suppress responses altogether.
Methods

Animals

We used Thy1-GCaMP3 (Chen et al., 2012) male mice (8-14 weeks old). Animal care and experiments were approved by the Hebrew University Animal Care and Use Committee.

Surgical procedures

We anesthetized mice with an intraperitoneal injection of ketamine and medetomidine (100 mg/kg and 0.83 mg/kg, respectively) and a subcutaneous injection of Carprofen (0.004 mg/g). Additionally, we injected mice subcutaneously with dextrose-saline to prevent dehydration. We assessed the depth of anesthesia by monitoring the pinch withdrawal reflex and added ketamine/medetomidine to maintain it. We continuously monitored the animal’s rectal temperature and maintained it at 36°C ±0.5°C. For calcium imaging, we made a small incision in the animal’s skin, and glued a custom-made metal bar to the skull using dental cement in order to fix the head for imaging under the microscope. For acute imaging we performed a craniotomy (2x1mm) over the olfactory bulb of one hemisphere. We placed 1.5% low melting agar (type IIIa, Sigma-Aldrich, St. Louis, MO) over the craniotomy covered by a glass cover which was then secured with dental cement. For awake experiments a single 3 mm round craniotomy was opened over the OBs of both hemispheres using a 3 mm biopsy punch (Miltex), and the bone was carefully removed. The exposed brain was covered directly with a 3-mm-diameter round cover-glass (Menzel-Glaser). The margin between the cover-glass and the intact bone was gently sealed with Histoacryl glue (B. Braun). After surgery, mice were treated with carprofen (0.004 mg/g, s.c) until full recovery. All animals were allowed to fully recover before the first imaging session, which started at least 2 weeks after surgery.

Two-photon calcium imaging

We performed calcium imaging of the OB using an Ultima two-photon microscope from Prairie Technologies (Middleton, WI), equipped with a 16X water-immersion objective lens (0.8 NA;CF175, Nikon). We delivered two-photon excitation at 950nm using a DeepSee femtosecond laser (Spectraphysics). The size of an imaging field was 169 X
169 μm (420 X 210 pixels). Acquisition rate was ~7 Hz. Before awake imaging, and two
weeks after implanting the window we habituated the mice under the microscope in the
head fixed position. Each mouse was habituated once a day for 15 minutes for 4 days.
Awake imaging was performed in habituated mice, which showed no obvious sign of
stress.

**Odor delivery**

To deliver odorants we used a custom-made olfactometer. In order to avoid cross-
contamination between odorants we used separate tubing for each channel, from the odor
vial to the animal’s nose. We used a panel of 7 odorants (ethyl-acetate, methyl-
propionate ethyl-tiglate, butanal, ethyl-butyrate, isoamyl acetate and propanal; all
obtained from Sigma-Aldrich) and one blank channel with no odor inside. We presented
odorants in a final concentration of 250 ppm at a rate flow of 1 l/min. The short stimuli
lasted 2 seconds with a 15 second inter-stimulus interval. The target-over-background
stimuli lasted 50 seconds long with a 60 seconds inter-stimulus interval. We repeated all
stimuli 4 times in a pseudo-random order. In order to equalize flow rates and
concentrations between the different stimuli, we delivered each target odor at 500 ml/min
together with a 500ml/min blank stimulus and mixture stimuli at 500ml/min for each
odor. In the target-over-background protocol we delivered the background odor together
with the blank (500ml/min for each). When target-over-background was presented, the
blank stimulus was turned off and the target stimulus was turned on. Odor delivery was
monitored using photoionization detector (mini PID from Aurora). In a separate set of
experiments, we repeated the same protocol but with 10-fold lower odor concentration
(25 ppm).

**Data analysis**

We analyzed all data using custom written code in Matlab (The Mathworks). Regions of
interest corresponding to individual cell bodies were manually drawn and the mean
fluorescence of each cell body was extracted by ImageJ and exported to Matlab for
analysis. Relative fluorescence change (Δf/f) was calculated as follows: baseline
fluorescence (f0) was the mean fluorescence over 2 second before odor onset. Traces
were low-pass filtered using a square filter with a 3-sample window. Zero-phase filtering was achieved by two passes of the filter using the Matlab filtfilt function. In awake imaging data, small lateral movements were corrected using cross correlation image alignment. In a few cases (less than 1% of all responses) the responses were calculated based on 3 trials (instead of 4). Coefficient of variance was calculated as the variance of peak amplitudes between each trials for all MCs divided by their mean. Decay times were calculated by an exponential fit from the peak amplitude to steady state using the ‘fit’ function in Matlab.

Principal component analysis (PCA) was performed considering the entire recording period from the beginning of the odor stimulation to the end of recording for all different stimuli of all trials. To quantify distances between responses we created response vectors from all cells measured in each individual mouse. These vectors were composed from the mean Δf/f response values of the neurons at peak amplitudes for the solo and mixture odors (2 seconds after stimulus onset) and the same time after target-over-background onset (42 seconds). Each vector was normalized to the number of MCs in each mouse, and the distance was calculated using the Euclidean distance between the vectors. Classifications were done using the Matlab function 'TreeBagger' (200 trees).

Each training stimulus was a vector composed of 4 trials at the peak amplitude of the stimulus (2 seconds after stimulus onset). Test trials were composed from the mean of 4 trials at the average time of peak amplitudes (2 seconds for single odors and mixtures, and 42 seconds for target-over-background). Shuffled data was created by shuffling the test stimuli across neurons for the same stimulus.

**Experimental Design and Statistical Analysis**

We recorded calcium transients in the OB of anesthetized and awake mice. Calcium transients were categorized as odor evoked responses if all trials in addition to the mean had 3 consecutive Δf/f values within the response window that were found to be above the mean +1.6 s.d of the values in the blank trial. Only cells that were responsive to at least two odors were included in the single cell response analysis (Fig.3,4,5). We used an unpaired two-sample t-test to compare between the solo responses to the target-over-background and the t(B)-baseline (non-parametric tests yielded similar results). All
comparisons were done between the time bin of the averaged peak in the solo protocol and the equivalent time bin of target stimulus in the target-over-background protocol. Mixture changes were measured between the average peak amplitude of each component and the peak amplitude of their mixture. To calculate adaptive responses, we compared the maximum values of 4 trials at the beginning of the background stimulus (first 5 seconds) to the maximal values just before the target onset (35-40 seconds) using unpaired two sampled t-test. Responses that were significantly lower at 35-40 were considered suppressed and classified as ‘adapting’. Significantly higher trials were classified as ‘increasing’ and those that were not significantly different were classified as ‘no change’. We used population analysis to counter the effect of bias the analysis to responsive neurons.
Results

We used in vivo two-photon calcium imaging to test how MCs respond to a continuous odor as background, and how odor coding is affected by the short history of this background activity. We imaged the activity of MCs in Thy1-GCaMP3 mice expressing the genetically encoded calcium indicator GCaMP3 in MCs (Chen et al., 2012). MCs were identified morphologically by their large somata at ~250-300 microns under the surface of the brain (Fig. 1A,C,E). To evaluate MC activity, we used 7 monomolecular odors and their mixture pairs known to activate the dorsal surface of the OB: ethyl-acetate (Ea), methyl-propionate (Mp), ethyl-tiglate (Et), butanal (Bu), ethyl-butyrate (Eb), isoamyl acetate (Ia) and propanal (Pr)(Adam et al., 2014; Livneh et al., 2014).

We focused on a protocol in which one odor becomes ‘background’ due to its prolonged presence and another odor is considered ‘target’ because it is transiently presented on top of the prolonged background odor (Kadohisa and Wilson, 2006; Gottfried, 2010; Saha et al., 2013). For each odor pair the full protocol included 5 stimuli – two presented alone (diluted by air only and referred herein as - “solo” odors), their binary mixture, their target-over-background combinations, (where the odor indicated in parenthesis corresponds to the background) and a blank stimulus (Fig. 1B,D,F). We first tested responses to a continuous background odor alone. Then, we examined the responses to target-over-background and compared these to responses of the target stimulus without background (‘solo’).

The target-over-background stimuli were composed of 50 seconds ‘background’, and a 2-sec ‘target’ starting 40 seconds after the initiation of the background stimulus (Fig. 1B). A total of 10 odor pairs were examined in the target over background protocol (Fig. 1B,D,F). In each imaging field, we collected data from a few dozen MCs simultaneously (average ± sem: 34.9±2.3MCs). Representative examples showing a single field of imaging and responses from 4 MCs are shown in figure 1A,B for the odors Ea, Mp, and Et. Additional examples from different fields and odors are shown in figure 1C-F.
**Background suppression in the OB**

We started by imaging anesthetized mice where we collected responses from many MC’s under stable physiological conditions. To increase the number of cells responding to two or more odors, we initially used a relatively high odor concentration (250 ppm) and collected data from 1325 MCs (n=10 mice). Under these conditions, 1453 cell-odor pair responses to background were measured. Importantly, odor concentration during delivery remained largely stable across the 40 seconds stimulation of background odors (Fig. 1G).

Calcium responses to background odor stimulation were frequently adaptive. The mean initial calcium response peaked at around 2 seconds after stimulus onset and receded shortly thereafter (Fig. 2A). 15 seconds after stimulus onset, mean responses were already significantly lower, reaching roughly 50% of their initial peak (0.34±0.01 Δf/f at the peak, 0.18±0.01 at 15 sec p<0.001, two-sided Wilcoxon signed rank test). This decrease remained stable at 40 seconds after stimulus onset (which is just prior to the time when the target odor was presented; Fig. 2A; 0.16±0.01 at 40 sec). Despite the significant trend of the general adaptation, individual MC responses were heterogeneous (Fig. 2B). On average 71.3% of the responses adapted per odor during the continuous background odor (Fig. 2B- blue), 18.3% remained unchanged after 40s of background (Fig.2B - red), and 10.4% showed gradual increase (Fig. 2B- black). The adaptation of a response was positively correlated with the strength of the initial response to the background. Stronger initial responses were adapted strongly and weak responses showed little adaptation or even a gradual increase (Fig. 2C- each odor individually; 2D- all odors combined).

**MCs responses to target over background**

Using the same dataset described above (n=1325), we next asked to what extent MC adaptation affects responses to additional odors when these odors appear on top of background. We refer to these new odors as “target-over-background (t(B))” or simply as ‘target’. To study how background affected individual MCs responses we analyzed MCs responding to both background and target odors. Out of the 1325 MCs recorded, 430
MCs responded to both background and target odors for a total of 1258 cell-odor pairs. Solo odors showed a range of response magnitudes (Fig. 1B,D,F; Fig. 3A,B - red and blue traces). Target-over-background \((t(B))\) responses were heterogeneous as well (Fig. 3A,B - black traces). We, therefore, classified these responses to two categories with reference to the solo odor: 1) Target-over-background responses different than the solo (Fig. 3A: \(t(B) \neq \text{solo}\)), and 2) Target-over-background responses not significantly different than the solo (Fig. 3A: \(t(B) = \text{solo}\), right). We first compared \(t(B)\) responses to the absolute level of the solo response (Fig. 3A, red vs black). The distribution of this comparison varied widely and was to a large extent odor-specific (Fig. 3C). Pooling all odors together, 58% of responses were classified as being similar in both conditions (i.e. \(t(B) = \text{solo}\); Fig. 3E, grey). Different fields from the same mice contributed similarly to the distribution of values between solo and \(t(B)\) (data not shown).

We also defined the relationship between the solo and \(t(B)\) responses differently, and considered the immediate background level preceding the stimulus as the baseline (Fig. 3B – blue vs black, referred to as \(t(B)-\text{baseline}\)). Calculating the comparison this way, again, shows that the distribution of values was odor-specific (Fig. 3D). Pooling all odors together using this method, 44% of responses were classified as being similar to the target in both conditions (i.e. \(t(B)-\text{baseline} = \text{solo}\); Fig. 3F, grey). Comparing the two methods side by side showed a strong correlation between the two, strengthening the claim that target responses are odor-specific (Fig. 3G). Note that in a subset of the odor pairs, the solo and target odors were not different in over 50% of the cases in both measures (Fig. 3C,D – grey in Ea(Mp), Et(Ea), Mp(Ea), Ea(Et), Eb(Bu)). Thus, in these odor pairs, the presence of background did not seem to affect the identity of the majority of odors (see more below). The responses to these odors suggest that while the system suppresses background it maintains the ability to identify an odor in face of background.

Given the heterogeneity of responses, one possible explanation for our results could be that the level of adaptation determines the level of similarity between an odor with background and without background. For example, a highly-adapted response could “reset” the system to basal conditions, as if no background were present at all. To test this, we plotted the level of adaptation of each neuron versus how solo and \(t(B)\) compare.
We found no obvious relationship between the level of adaptation and how similar the odor would be with or without a background (Fig. 3H). This result rules out a simple explanation whereby background suppression “resets” the system to detect an odor over background as anew. Thus, residual activity in the OB during odorous background conditions, does impact how new odors are processed.

Mixture processing is different in the face of background

In our protocol, the stimulus during target-over-background is the exact same stimulus as the binary mixture. The only difference between stimuli is the preceding background history. We thus compared how MCs responded to mixtures with regard to their responses to the mixture components with and without the history of background. To do so, we first analyzed the change MC responses undergo in the transition from a single odor into a mixture without background. This analysis is exemplified in one representative MC responding to an odor mixture (‘Ea+Et’) as well as to the single odor components in isolation as solo odors (‘Ea’ and ‘Et’) (Fig. 4A, No Background). The fluorescence change from the response to ‘Ea’ into a mixture is evident as a stronger response to the mixture (Fig. 4A; quantified as ‘mixture change’=0.17 Δf/f). However, the change from the response to ‘Et’ into the mixture is evident as a slightly weaker response to the mixture (Fig. 4A; ‘mixture change’ =-0.04 Δf/f). In both cases, the mixture response was smaller than the sum of the responses to the components. This phenomenon is known as ‘mixture suppression’, which is very well documented in olfaction (Giraudet et al., 2002; Kadohisa and Wilson, 2006; Davison and Katz, 2007; Stettler and Axel, 2009; Shen et al., 2013).

We next tested whether mixture changes were themselves affected by background history. We utilized the analysis shown above in figure 3B, which is the same as measuring the ‘mixture change’ in the presence of background. In the example of figure 4A, response-changes to the mixture Et(Ea) were larger in the ‘No Background’ condition as compared to the “With Background” condition (Fig. 4A; compare ‘Et(Ea)-baseline’=1 Δf/f to ‘mixture change’=0.17 Δf/f). Similarly, the change in Ea(Et) was
larger than the ‘No Background’ condition (compare ‘Ea(Et)-baseline’=0.4 Δf/f to ‘mixture change’=-0.04 Δf/f). Thus, the exact same chemical stimulus (i.e. ‘Ea+Et’, ‘Et(Ea)’, ‘Ea(Et)’) induced different responses depending on the identity of the preceding stimulus history (i.e., ‘no background’, ‘Ea’ as background, or ‘Et’ as background). Further, a side by side comparison of response changes to mixtures with and without background showed no correlation (Fig. 4B), strengthening the claim for a history-dependence of MC responses.

To study more carefully the single odor responses versus mixture responses under different background conditions, we looked for factors that were correlated with the mixture suppressive behavior of MCs. In the “No Background” condition stronger responses to an odor were negatively correlated with the resultant mixture suppressive effect. Stronger responses to a single odor were accompanied by higher suppression in its mixture condition (Fig. 4C, black vs. green; y=-0.3x+0.3, r=-0.2). In contrast, when the exact same odors were preceded by background, the same neurons were no longer so mixture-suppressive (Fig. 4D; black vs. blue; y=0.5x+0.3, r=0.3). Notably, target-over-background responses were not only odor-specific but also background specific. The responses of MCs were different depending whether an odor was background or target. In the most extreme odor pair, MCs responses showed either no response to one odor as a target (Fig. 4E; Bu(Eb)) but remained sensitive to the other odor (Fig. 4E; Eb(Bu); Fig. 3D - compare grey dots in Eb(Bu) vs Bu(Eb)). Taken together, these data show that mixture processing by MCs strongly depend on the preceding history of the odor and its identity.

Background adaptation and target responses over background are similar in the awake state and in lower odor concentrations

The results described thus far have been obtained by imaging MCs in anesthetized mice. Experimental conditions in anesthetized mice have some advantages over the awake state such as being experimentally stable both mechanically and physiologically (Adam et al., 2014). Nevertheless, there are obviously physiological differences between the awake and anesthetized states. For example, in the OB of anesthetized mice, MCs were shown to
have significantly stronger responses due to an attenuation of normal granule cell inhibition (Rinberg et al., 2006; Kato et al., 2012; Cazakoff et al., 2014). Thus, we next carried out the exact same experiments in 4 out of the 10 odor combinations in head-restrained awake mice.

We implanted mice (n=3) with a chronic window over the OB and imaged MCs two weeks after window implantation (Adam and Mizrahi, 2011; Adam et al., 2014). During imaging, mice were held in an awake, head-fixed position under the microscope as described by others (Kato et al., 2012). We measured calcium responses from MCs to the high odor concentration of 250 ppm (Fig. 5A, B). As reported before, in awake mice calcium responses were weaker and less MCs were responsive. Out of 186 imaged cells, only 43 cells were further analyzed (for a total of 112 cell-odor pairs).

Despite the weaker (Fig. 5C; Peak amplitudes - anesthetized:0.24±0.01, awake:0.17±0.01, p=1.8x10⁻⁸; t-test), noisier (Fig.5D; Coefficient of variance – anesthetized:0.35±0.02, awake:0.65±0.2, p=9.6x10⁻⁸; t-test) and fewer responses of MCs in awake mice (responsive cells- anesthetized:84% awake:64%), the results were qualitatively similar to the data collected from the anesthetized mice (one exception is a slightly faster average response in the awake state; Fig.5E; response decay time – anesthetized: 9.0 seconds, $R^2 = 0.99$, awake:6.6 seconds $R^2 = 0.82$, p=0.04; t-test). On average, MCs adapted to roughly 60% of their initial value of the background stimulus (anesthetized from 0.24±0.01 at the peak to 0.14±0.01 at 40 seconds; awake from 0.17±0.01 to 0.11±0.01 at 40 seconds). Single MC analysis revealed that 51.4% of responses adapted, 25.4% remained similar and 23.2% increased (Fig. 5F). Adaptation values were correlated strongly to the intensity of the background (Fig. 5G), and most (87.5%) responses to target-over-background did not significantly differ when presented after 40 seconds background (Fig. 5H). Differences from the anesthetized state (e.g. less neurons passing significance from the diagonal) could also be due to the noisier nature of the weaker responses. Taken together, these data show that background adaptation and sensitivity to target odors over odorous backgrounds are qualitatively similar in awake and anesthetized mice.
To test the contribution of odor intensity to target sensitivity and adaptation, we repeated the same experiments using 10-fold lower odor concentration (25 ppm). As expected, less MCs were now responsive (59%), but odor evoked responses had similar coefficient of variance values (0.31±0.02) and decay dynamics (response decay time – 9.4 seconds, $R^2=0.99$; similar to the 250 ppm, p=0.36 but significantly different from the awake, p=0.03; t-test). Moreover, both background and target-over-background responses were highly similar (Fig. 5F-H –‘25ppm’). We conclude that our results of both background adaptation and target responses remain across physiological states and odor concentrations.

**Population coding is history-dependent**

Thus far, we only analyzed response properties of single neurons, which were responsive to a pair of the odors tested. Coding, however, is carried out by the collective activity of multiple neurons, many of which have variable and overlapping response profiles. For example, some neurons in our dataset responded to all three odors (Fig. 1B, cells 1-4), two odors (Fig. 1F, cell 2), one odor (Fig. 5B, cells 1,3), or none (Fig. 1F, cell 3). Considering the whole population of neurons, we asked how does the population encode odors collectively with reference to recent background history. An example of all cells and all responses imaged from mice tested with the odor pair Ea-Mp are shown in figure 6A,C.

As expected from the single neuron responses, population responses to odors ‘Ea’ and ‘Mp’ in isolation were different (Fig.6A; compare two left panels, red/green arrows). To evaluate how similar/different these population responses are, we used Principal Component Analysis (PCA). For odors ‘Ea’ and ‘Mp’, PCA analysis shows distinct trajectories on the 2-second stimulus peak (Fig. 6B, red and green, respectively). Next, we assessed and found that the population response to an odor changed under different conditions of background history. For example, the population response of odor ‘Ea’ on top of a ‘Mp’ background (‘Ea(Mp)’), was different than the response of odor ‘Mp’ on top of an ‘Ea’ background (‘Mp(Ea)’) (Fig. 6C, D). The difference is evident both in the
raw traces (Fig. 6C, compare responses above black arrows) and in PCA space (Fig. 6D).

Importantly, both conditions were different from the population response to the binary mixture ‘Ea+Mp’ under the ‘no background’ condition (Fig. 6D; compare black to magenta).

To quantify the effects observed in the PCA analyses, we measured each population response as a multi-dimensional vector for each odor and calculated the distance in population responses between the two vectors (see Methods). Specifically, we measured the distance in population responses between the ‘mixture’ stimuli under different background conditions. For example, for the Ea and Mp odors we looked at any pairwise mixture combination (‘Ea(Mp)’, ‘Mp(Ea)’, ‘Ea+Mp’), versus the 2 different odors Ea vs Mp in isolation. On average, pooling all odors together, odor mixtures with different backgrounds showed distances of ~0.2 Δf/f and these were as distant as pairs of different odors in isolation (Fig. 6E; two left bars; p>0.05, Wilcoxon rank-sum test). Importantly, almost all individual comparisons yielded similar distance values (with one exception for the odor pair Ia and Mp). Thus, the responses to the exact same odor mixture under different background conditions was as different as two different odors. We conclude that population responses to mixtures in the OB show strong history-dependence.

Impact of history-dependent responses

Next, we tested to what extent the MC population can detect an odor as such under continuous background activity. Since this quality was odor-specific at the single cell level (Fig. 3), we tested this by odor at the population level as well. When we limited our analysis only to those subsets of odors that showed stability in>50% of responses (i.e. Ea(Et), Et(Ea), Ea(Mp), Eb(Bu) and Mp(Ea) in Fig. 3C,D), population responses to the target were similar to the isolated odor (Fig. 6F – compare Et to Et(Ea) in raw traces and in the PCA). The distance between population response for a solo odor relative to its response as a target was smaller by~ 30% as compared to the differences between different isolated odors (0.15Δf/f ± 0.01). Notably, the ability of the system to maintain odor similarity in face of background was odor-specific. Other odors (e.g. Bu vs Bu(Eb))
did not induce a target response at all, in face of the highly dominant Eb background (Fig. 6G). Similarly, other odor pairs like Mp(Pr) and Mp(Ia)) were dominated by background, and showed a weak target response.

Finally, we evaluated how a downstream decoder would classify MCs responses to isolated odors and on top of background. We trained a classifier (Decision tree, see methods) on one pair of odors and then tested its classification decisions on different stimuli composed from these odors. Figure 7A show results of the classifier after training MCs on the pair of odors ‘Ea’ and ‘Mp’ (raw traces of these odors are shown in Fig. 6A,C). In each session, we randomly picked a number of MCs (from 1 to all imaged MCs, repeated 100 times) for training and testing. Classification accuracy for isolated odors was perfect for any population larger than 5 MCs (Fig. 7A, left). When the same MCs were tested on the mixture stimuli, which were not used for training, more MCs were needed for the decoder to reach clear classification (Fig. 7A, right). For this odor pair, the classifier correctly classified the target as such when the target was presented over background. For example, when ‘Mp’ was the background and ‘Ea(Mp)’ the target, the classifier identified the mixture as ‘Ea’ (Fig. 7A, Ea(Mp)). Similarly, when ‘Ea’ was the background, and ‘Mp(Ea)’ the target it was now identified as ‘Mp’ (Fig. 7A; See also their PCA projections in Fig. 7B). This phenomenon was robust in all those odor combinations that remained sensitive to the target odor in >50% of their responses (Table 1). This result also remained similar in the low odor concentrations, and in awake mice (Table 1). These results show that for a majority of odor pairs, when an odor is presented over-background, it is identified by the classifier as the “target” odor and not as the background.

As mentioned above, some target odors induced rather weak responses when presented over background (Fig. 3D – Bu(Eb), Mp(Ia), Mp(Pr)). In those odors, background dominated the responsivity of the network, masking additional responses (Fig. 7C,D compare Mp to Mp(Ia)). In these cases, a decoder trained on the single odors often made mistakes in identifying the t(B) mixtures as the correct target odor (Fig. 7E; see decoder performance on Mp(Ia)). For those dominant background odors, further processing beyond the OB would be needed to change background activity to a level that
will allow a reliable detectable of a target. Alternatively, these background odors could be
difficult to detect perceptually under these conditions.
Discussion

We imaged odor-evoked activity of MCs to continuous stimuli as well as to target-over-background. The vast majority of MCs changed their activity levels during the prolonged odor presentation suggesting that odors are dynamically encoded in the OB at slow time scales. Despite some heterogeneity, the majority of MCs showed adaptation levels that are scaled by stimulus intensity. When a target odor was presented over an odorous background, MC response patterns were complex across all odors. Specific responses to target-over-background varied based on the identity of the odor but were always affected by the presence of background. This suggests that odor processing is dynamically updated in a history dependent context as early as the OB.

Background suppression and target sensitivity

The OB is not a simple relay station. Olfactory sensory neurons (OSNs) send odor input into the OB, which is then processed by several local inhibitory and excitatory networks (Wilson and Mainen, 2006; Murthy, 2011). Odor input is also modulated by centrifugal input or feedback connections projecting back into the OB (Shea et al., 2008; Petzold et al., 2009; Boyd et al., 2012; Ma and Luo, 2012; Markopoulos et al., 2012). As a result, MCs output is a transformed version of the initial odor input (Kikuta et al., 2013; Adam et al., 2014). Accumulating evidence focusing mainly on short time scale responses suggest that MCs output may reflect numerous computations like pattern de-correlation (Padmanabhan and Urban, 2010; Friedrich and Wiechert, 2014), contrast enhancement (Cleland, 2010), normalization (Cleland et al., 2007; Olsen et al., 2010) and even “higher” computations like those that reflect odor value (Doucette and Restrepo, 2008) or animal state (Pirez and Wachowiak, 2008). We present a description of single cell and population response profiles of MCs with regard to how they process odor input at slow time scales.

The responses we observed in MCs are potentially a result of upstream, local, or downstream computations. The initial candidates for shaping MC adaptive responses are the OSNs, located upstream in the hierarchy. Indeed, the initial phase of background
suppression most likely carries a strong OSN component, which show adaptation both in vitro and in vivo (Zufall and Leinders-Zufall, 2000; Bradley et al., 2005; Kleene, 2008; De Palo et al., 2013). For example, Lecoq et al. (Lecoq et al., 2009) studied adaptation to 4 sec stimuli and found that the adaptation in glutamatergic axons of OSNs can explain glomerular adaptation as measured by local field potentials suggesting that the OSN-MC synapse is the sole locus responsible for this adaptation. Our measurements were different than those of the Lecoq et al. study both in the duration of the stimulus (here, 40 sec) and in the location of measurement (i.e. MC somata). Thus, while the initial adaptive phase in the glomerulus is most likely explained by OSN adaptation, the developing responses in MC somata along the 40 sec stimulus would involve other downstream synapses which are most certainly active. In the fly, Carafo (Cafaro, 2016) made direct measurements of adaptation from projections neurons (PNs, the fly analogue of MCs) alongside with their cognate OSNs. He found that PN adaptation is greater than OSN-adaptation, and described additional sources for adaptation downstream of OSNs. Notably, the responses of MCs that we measured were not unanimously adaptive; 28% of neurons did not adapt and significant portions of these increased their responses. The anatomical complexity of OB circuits downstream of the OSN-MC synapse is thought to be more complex in the mouse than in the fly, and remain likely candidates to contribute to MC response profiles. Additionally, OSNs responses to mixtures are often reported as cumulative (Lin et al., 2005; Verhagen et al., 2007; Fletcher, 2011, Saha et al. 2013; but see Duchamp-Viret 2003; Rospars et al., 2008, Su et al., PNAS 2011), while MCs responses to mixtures are suppressive (Fig. 4, (Giraudet et al., 2002; Davison and Katz, 2007)). Thus, mixture stimuli most likely recruit additional mechanisms (e.g. inhibitory circuits) to suppress MC activity to mixtures. Future studies await a more mechanistic explanation of how the slow responses from different circuit components develop in the OB.

There are several other candidate subpopulations that provide inhibitory (and excitatory) input to MCs and could further contribute to the effects we observe. For example, both intra-glomerular and inter-glomerular inhibition is known to shape the amplitude and temporal activity of odor responses (Shao et al., 2012; Kato et al., 2013; Miyamichi et al., 2013; Banerjee et al., 2015). These could potentially contribute to the suppression of continuous background odors in an odor-specific manner. Granule cells
are also locally active and could contribute to achieve specificity (Arevian et al., 2008; Koulakov and Rinberg, 2011; Kollo et al., 2014). Given its slow time to evolve, sources of adaptation are not necessarily fast, nor direct. Thus, downstream sources like cortical feedback are well positioned to play a role in MC adaptation (Boyd et al., 2012; Markopoulos et al., 2012). Recently, feedback projections were shown to mediate odor specific effects on MCs; a property that could serve as a mechanism for odor specific MC adaptation (Boyd et al., 2015; Otazu et al., 2015). Adaptation could involve numerous inhibitory sources that operate simultaneously and or consecutively. For example, granule cells may be activated by centrifugal feedback and indirectly suppress MCs (Koulakov and Rinberg, 2011). In fact, centrifugal feedback as a mechanism for adaptation has been proposed already 25 years ago by modeling studies (Li, 1990), yet with little empirical support.

### History dependent processing - behavior implications

In nature, odor stimuli are composed of complex mixtures. However, here we used pure odorants that are neither behaviorally Meaningful nor complex. Behavioral correlates of odor sensitivity over background using simple mixtures have been described in the past. For example, Linster and colleagues trained rats to associate a simple odor with water reward (Linster et al., 2007). Rats were then adapted to a prolonged constant background odor resulting in a decrease of their behavioral response to the background. Rats remained sensitive to new odors presented on top of the adapted background showing that adaptation, even to simple mixtures, impacts perception. Projection neurons activity in the locust antennal lobe (analogous to the OB in insects) have also been found to correlate with perception of target odors over background in an odor dependent manner (Saha et al., 2013). MC activity has been shown to be modulated by experience (Kay and Laurent, 1999; Mandairon and Linster, 2009) and to encode odor value (Doucette and Restrepo, 2008; Restrepo et al., 2009; Doucette et al., 2011). We speculate that behaviorally meaningful odors would have higher sensitivity in face of background. Exploring whether naturally meaningful (aversive or attractive) odors or learned odors
share similar properties to simple odors could shed light on the mechanisms that support these computations in the OB.

The rich behavioral repertoire of mice, like social recognition or their ability to locate foods and avoid predators in odor-rich environments, would rely heavily on segregating salient odors from background. In real life, where mixtures are complex, we expect even stronger adaptation as more inhibitory channels are activated. Identifying neural correlates of background suppression and history dependent processing so early in the olfactory hierarchy sets a substrate for higher computations performed in cortical regions like, for example, object recognition in complex odor scenes.
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Figure legends

Figure 1: The experimental protocol. A, In-vivo two photon micrograph showing a field of view of GCaMP3 expressing mitral cells at 267μm below the dorsal surface of the main OB. Scale bar: 50 μm. B, Representative examples of odor responses from four MCs in ‘A’. The odor stimuli used are Ea-Ethyl-acetate, Mp-Methyl-propionate, Et-Ethyl-tiglate, mix - binary mixtures, t(B) indicates the target-over-background protocol where ‘t’ is the target and ‘B’ is the background. The target odor is delivered starting at 40 seconds after background onset. Asterisks indicate statistically significant responses. Scale bars - 50% Δf/f and 10 seconds. C,D,E,F, Additional examples from different mice using different odors. Details as in ‘A’ and ‘B’ for the following odors: Bu-Butanal, Eb-Ethyl-butyrate, Ia-Isoamyl acetate, Pr-Propanal. G, Photoionization detector traces of the full protocol for two odors in isolation (‘Ea’ and ‘Mp’), their mixture (‘Ea+Mp’) and the target-over-background stimuli (‘Ea(Mp)’ and ‘Mp(Ea)’). Inset – a zoom in on the traces comparing the mixture and the two target-over-background conditions. Note that the mixture and the target-over-background show identical traces and no deleterious effects of our olfactometer.

Figure 2: MCs adapt to background in an intensity dependent manner. A, MCs responses to 7 different background odors (duration – 40 sec). Black-Mean traces, grey-sem. B, Same responses as in ‘A’ only separated to adapting (blue), no change (red) and rising (black). C, Linear correlations between background intensity at the peak (peak at 2 seconds, x-axis) and after background change (peak at 2 seconds – pick at 40 seconds, y-axis), per odor. Black curves reflect the linear regression equation on the top. Dotted line here and throughout the rest of the manuscript is when x=y. D, Linear correlations between background intensity and background change, combined for all odors.

Figure 3: MCs responses to target-over-background. A, Representative examples of calcium responses to the target-over-background stimuli (black traces, drawn with reference to f(0)) as compared to solo stimulus (red, solo response, drawn with reference to f(0)). B, Representative examples of calcium responses to target-over-background stimuli (black traces, drawn with reference to f(0)) as compared to the solo response (blue, drawn with reference to the background level prior to target onset). C, Scatter plots
for all odors separately comparing the maximum calcium response of the solo (x-axis) to its response as a target-over-background (t(B); y-axis). Each data point is one cell-odor pair. Red dots and red numbers are responses where t(B)≠solo (unpaired two-sample t-test, p<0.05), and grey dots and black numbers represent t(B)=solo. D, Same as C but the t(B) response is calculated from background baseline rather than f(0) (t(B)-baseline; y-axis). E-F, Same as C-D but for all odors combined. G, Correlation between t(B) when amplitude is measured from f(0); t(B), x-axis) and t(B)-baseline (when amplitude is measured from the baseline of background (t(B)-baseline, y-axis). Black curve reflects the linear regression equation on the top. H, t(B)-baseline responses (t(B)-baseline, y-axis) versus the level of adaptation to the background odor prior to target onset (Background change, x-axis).

**Figure 4: Mixture processing is modulated in the face of background.** A, Representative example of a MC, which was more sensitive to a target odor when background odor was present. The traces show the responses of this MC to 5 stimuli (‘Ea’, ‘Et’, ‘Ea+Et’, ‘Et(Ea)’ and ‘Ea(Et)’). The grey dotted lines are references to baselines and response amplitudes denoting the changes in activation from one state to the next (arrows). For example, the transition from ‘Ea’ to ‘Ea+Et’ induces a 0.17Δf/f change in fluorescence (‘mixture change’, bottom green to black line). However, the response during background to the exact same stimulus was a change of 1Δf/f (‘t(B)-baseline’, blue to black line in the (Et(Ea) responses). Similarly, the transition from ‘Et’ to ‘Ea+Et’ was a small decrease of -0.04Δf/f while a 0.4Δf/f increase was observed when background was present (Ea(Et)). B, Left- scatter plots of t(B)-baseline for all odors sorted by their amplitude (blue) and the mixture change for the same odor combination in the same cell (green). Right- Plot of the correlations between ‘mixture change’ (x-axis) and the change for the same mixture but when one odor is the background (t(B)-baseline, y-axis). C, Scatter plot of responses to the ‘solo’ odor (black; sorted by amplitude) and the change in the mixture response by the same cell (green). Higher responses have lower mixture change (y=-0.3x+0.3, r=-0.2). D, Same as ‘C’ but blue is the ‘t(B)-baseline’ response. Here, higher responses do not show negative but rather more positive response.
changes \((y=0.5x+0.3, \, r=0.3)\). E,F Same as in 'D' but only for the odors Bu and Eb. In ‘E’ Bu versus Bu(Eb) \((y=-0.04x+0.01, \, r=-0.26)\), and in ‘F’ Eb versus Eb(Bu) \((y=0.36x+0.17, \, r=0.43)\). Note that background effects are not symmetric. See main text for details.

**Figure 5:** MCs responses to target-over-background in low concentration and awake mice. A-B. Similar to Fig.1 A-B but in head restrained awake mice. C, Average of peak amplitude responses in awake and anesthetized mice. ***p=9.6x10^{-8}, t-test. D, Average of the coefficient of variance (COV) values in awake and anesthetized mice. ***p=1.8x10^{-8}, t-test. E, Average decay time from peak amplitudes to steady state in the awake (red) and anesthetized (blue) states, normalized to the peak. *p=0.04, t-test. Grey – SEM between different mice. F-H, Side by side comparison of various response values using the same odor set to three different experimental conditions. Top- 250 ppm (anesthetized), middle - awake mice and bottom - 25 ppm (anesthetized). F, Average traces of the adapting (blue), the stable (red), and the increasing (black) MCs responses along a 40 sec background stimulus. G, Linear correlations between background intensity at the peak (peak at 2 seconds, x-axis) and after background change (peak at 2 seconds – pick at 40 seconds, y-axis). H, Top - Scatter plot comparing the maximum calcium response of the target alone (solo; x-axis) to the target (t(B); y-axis). Each data point is one cell-odor pair. Grey- Responses are not significantly different, Red - Responses are significantly different (unpaired two-sample t-test, \(p<0.05\)). Middle – same for awake mice. Bottom - 25ppm concentration. On top of each graph are the percentage of grey dots from the total number of responses.

**Figure 6:** Population analysis of MCs responses to single odors and mixtures in face of background. A, Full response profiles of 434 imaged cells to single odor stimuli (‘Ea’, ‘Mp’), and their binary mixture (‘Ea+Mp’). The stimulus is indicated above each plot by a black line. Color code is Δf/f. Time 0 is the beginning of odor stimulus. Arrows are at \(t=2\) seconds and they correspond to the time in ‘B’. B, PCA plots of two odor response vectors ‘Ea’ (red) and ‘Mp’ (green). Each dot represents a single trial from the
time point indicated by the arrow in '6A' (time at 2 seconds). C, Full response profiles of all 434 imaged cells to 'Ea(Mp)' and 'Mp(Ea)' odor stimuli. Black arrow corresponds to 6D at t=42 seconds. D, PCA plots for the 3 mixture stimuli with different backgrounds history. 'Ea+Mp' - No background history (magenta), 'Ea(Mp)'-history of 'Mp' as background (black), 'Mp(Ea)' -history of 'Ea' as background (black). E, Quantitative comparison of the Euclidean distances in PCA space for different pairwise comparisons where A and B represent different odors in a pair. Odor A vs Odor B – distance between 2 different solo odor responses. A+B vs A(B) or A+B vs B(A) – distance between mixture with and without background (p=0.95, Wilcoxon signed rank test). A(B) vs B(A) – distance between mixtures with different backgrounds (p=0.51, Wilcoxon signed rank test). Error bar- s.e.m. F, Top - Full response profiles of all 434 imaged cells to 'Et' alone and 'Et(Ea)' odor stimuli. Black and blue arrows correspond to the PCA plots at the bottom. Each circle in the PCA plot is an individual trial. After 2 seconds 'Ea' as background is adjacent in PCA space to 'Ea' as solo. Later along the background (40 seconds), Ea differs in PCA space. At time 42, 2 seconds after target onset Et(Ea), responses are similar to Et in PCA space. G, Same as ‘F’ but for odors 'Bu(Eb)'. Response profiles from 398 Cells.

**Figure 7: Odor classification on top of background.** A, Decision tree classification performance for different stimuli. The classifier trained on Ea vs Mp and tested on all 5 odor stimuli (as indicated on the left of each column). #MCs = 1,2,25,50,80,100,434. B, PCA plots for odors Ea and Mp as solo odors, as targets and as a mixture (same as in 6F,G). Note that Ea(Mp) is adjacent to Ea, and Mp(Ea) is adjacent to Mp. C, Left - Full response profiles of all 493 imaged cells to Ia and Mp as solo odors, and under background conditions. D, PCA plots of two odor response vectors Mp (red) and Ia (blue). Each dot represents a single trial from the time point indicated by the arrows in '7C'. While Ia(Mp) is adjacent to Ia in PCA space, Mp is distant from Mp(Ia). E, Same as in ‘A’ but for the odors 'Mp' and 'Ia'. Classification of 'Mp(Ia)' as 'Ia' is due to the dominance of 'Ia' as background.
Table 1: Response classification per field for odor response to Ea(Mp), Et(Ea), Mp(Ea), and Ea(Et) in awake and anesthetized mice. Fields with more than 25 MCs from all conditions were examined for stimulus classification (n=5 anesthetized 250 ppm, 4 awake and 6 anesthetized 25ppm). Test stimuli are indicated on the left column. Values are the average percentages of fields classified as one of the training stimuli.
Figure 3

A

B

c(B)/solo   c(B)/solo   c(B)/baseline/solo   c(B)/baseline/solo   c(B)/baseline=solo

C

Ea(Mp)   Mp(Ea)   Pt(Ea)   Ea(Ea)   Eb(Eb)   Bu(Eb)   In(Mp)   Mp(In)   Mp(Pr)   Pr(Mp)

D

E

All odors

F

G

H

r=0.7

y = 0.61x + 0.07

r = 0

Background change

(Δff [2 sec] × Δff [40 sec])
Figure 4

A. No Background (Mixture change)

B. Mixture change 

C. Solo Mixture change

D. Solo (t(B)-baseline)

E. Bu(Bu)-baseline

F. Eb(Eb)-baseline
Figure 7

A

Test stimuli

Training (Ea vs Mp)

Sluf. Ea Ea+Mp Ea(Mp) Mp(Mp)

1 2 5 100 MCs (60)

Classification as 'Ea' (%)

B

PCAl

Ea(Mp) Ea Ea+Mp

Mp(Mp) Mp(Mp)

0.05 0.05 0 PCA3

PCA2

C

Ia 1 3 5 10 12 15 20 25 30 35 40 45 50

#cell

49 3 2 1 0

D

PCAl

Ia Mp Mp+1a

In(Mp) In(Mp)

0.05 0.05

PCA2

E

Training (Mp vs Ia)

Test stimuli

Training (Mp vs Ia)

Shuf. Mp(Mp) Mp+1a

Ia(Mp) Ia(Mp)

1 2 5 100 MCs (#)

Classification as 'Mp' (%)
| Anes. 250ppm | Training | Training |
|--------------|----------|----------|
| Test         | Ea       | Mp       |
|              |          | Test     |
|              |          |          |
| Ea           | 100%     | 0%       |
|              |          | 100%     |
|              |          | 0%       |
|              |          |          |
| Mp           | 0%       | 100%     | Et      | 0%     | 100%   |
|              |          |          |         |         |
| Ea+Mp        | 60%      | 40%      | Ea+Et   | 80%    | 20%    |
|              |          |          |         |         |
| Ea(Mp)       | 80%      | 20%      | Ea(Et)  | 80%    | 20%    |
|              |          |          |         |         |
| Mp(Ea)       | 20%      | 80%      | Et(Ea)  | 60%    | 40%    |
|              |          |          |         |         |
| Awake        | Ea       | Mp       | Ea      | Et     |
|              |          |          |         |         |
| Ea           | 100%     | 0%       | 100%    |
|              |          | 0%       |
|              |          |          |
| Mp           | 0%       | 100%     | 0%      | 100%   |
|              |          |          |         |         |
| Ea+Mp        | 50%      | 50%      | 50%     | 50%    |
|              |          |          |         |         |
| Ea(Mp)       | 75%      | 25%      | 25%     | 75%    |
|              |          |          |         |         |
| Mp(Ea)       | 0%       | 100%     | 0%      | 100%   |
|              |          |          |         |         |
| Anes. 25ppm  | Ea       | Mp       |
|              |          |          |
|              |          |          |
| Ea           | 100%     | 0%       | 100%    |
|              |          | 0%       |
|              |          |          |
| Mp           | 0%       | 100%     | 0%      | 100%   |
|              |          |          |         |         |
| Ea+Mp        | 33%      | 66%      | 17%     | 83%    |
|              |          |          |         |         |
| Ea(Mp)       | 50%      | 50%      | 66%     | 33%    |
|              |          |          |         |         |
| Mp(Ea)       | 17%      | 83%      | 17%     | 83%    |

Table 1