Context- and Output Layer-Dependent Long-Term Ensemble Plasticity in a Sensory Circuit

YAMADA, Yoshiyuki, et al.

Abstract

Sensory information is translated into ensemble representations by various populations of projection neurons in brain circuits. The dynamics of ensemble representations formed by distinct channels of output neurons in diverse behavioral contexts remains largely unknown. We studied the two output neuron layers in the olfactory bulb (OB), mitral and tufted cells, using chronic two-photon calcium imaging in awake mice. Both output populations displayed similar odor response profiles. During passive sensory experience, both populations showed reorganization of ensemble odor representations yet stable pattern separation across days. Intriguingly, during active odor discrimination learning, mitral but not tufted cells exhibited improved pattern separation, although both populations showed reorganization of ensemble representations. An olfactory circuitry model suggests that cortical feedback on OB interneurons can trigger both forms of plasticity. In conclusion, we show that different OB output layers display unique context-dependent long-term ensemble plasticity, allowing parallel transfer of non-redundant sensory information [...]

Reference


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Context- and Output Layer-Dependent Long-Term Ensemble Plasticity in a Sensory Circuit

Highlights

- Mitral and tufted cells in the olfactory bulb show similar odor-evoked responses
- Passive odor experience reorganizes ensemble odor representations in both cell types
- Associative odor learning specifically improves pattern separation in mitral cells
- Cortical feedback can trigger both forms of plasticity in a network model

Authors

Yoshiyuki Yamada, Khaleel Bhaukaurally, Tamás J. Madarász, Alexandre Pouget, Ivan Rodriguez, Alan Carleton

Correspondence

ivan.rodriguez@unige.ch (I.R.), alan.carleton@unige.ch (A.C.)

In Brief

Yamada, Bhaukaurally et al. show that mitral but not tufted cells display improved pattern separation during active odor learning, while both display reorganized ensemble representations after passive and active sensory experience, enabling the olfactory system to perform multiplexed information processing.

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Context- and Output Layer-Dependent Long-Term Ensemble Plasticity in a Sensory Circuit

Yoshiyuki Yamada,1,2,5 Khaleel Bhaukaurally,1,2,5 Tamás J. Madarász,1,2 Alexandre Pouget,1,2,3 Ivan Rodriguez,2,4,* and Alan Carleton1,2,6,*

1Department of Basic Neurosciences, School of Medicine, University of Geneva, 1 Rue Michel-Servet, 1211 Geneva 4, Switzerland
2Geneva Neuroscience Center, University of Geneva, 1211 Geneva, Switzerland
3Gatsby Computational Neuroscience Unit, University College London, London, W1T 4JG, UK
4Department of Genetics and Evolution, University of Geneva, 1211 Geneva, Switzerland
5Co-first author
6Lead Contact

*Correspondence: ivan.rodriguez@unige.ch (I.R.), alan.carleton@unige.ch (A.C.)

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SUMMARY

Sensory information is translated into ensemble representations by various populations of projection neurons in brain circuits. The dynamics of ensemble representations formed by distinct channels of output neurons in diverse behavioral contexts remains largely unknown. We studied the two output neuron layers in the olfactory bulb (OB), mitral and tufted cells, using chronic two-photon calcium imaging in awake mice. Both output populations displayed similar odor response profiles. During passive sensory experience, both populations showed reorganization of ensemble odor representations yet stable pattern separation across days. Intriguingly, during active odor discrimination learning, mitral but not tufted cells exhibited improved pattern separation, although both populations showed reorganization of ensemble representations. An olfactory circuitry model suggests that cortical feedback on OB interneurons can trigger both forms of plasticity. In conclusion, we show that different OB output layers display unique context-dependent long-term ensemble plasticity, allowing parallel transfer of non-redundant sensory information to downstream centers.

INTRODUCTION

Collective activity of neuronal population, or neuronal ensemble representation, is proposed to be an important constituent of information processing in the brain. It has remained poorly understood, however, how the ensemble representation is maintained or modified over a long timescale (e.g., over days and beyond) by different types of output neurons. The recent advances in longitudinal and targeted large-scale recording with imaging offer the possibility to reliably track ensemble activity of identical neurons for a long time (Broussard et al., 2014; Lütcke et al., 2013; Rose et al., 2014). Although several studies have addressed the stability or the plasticity of ensemble representations in various brain regions in behaving animals (Andermann et al., 2010; Harvey et al., 2012; Huber et al., 2012; Kato et al., 2015; Komiyama et al., 2010; Peters et al., 2014; Poort et al., 2015), it still remains a hot topic of debate whether different populations of output neurons could display distinct forms of plasticity in diverse behavioral contexts (Chen et al., 2015; Li et al., 2015; Masamizu et al., 2014): when multiple groups of output neurons receive similar inputs yet project to different target regions, would they differently form and reorganize their ensemble representation depending on behavioral settings?

Here we addressed this question in the mouse olfactory bulb (OB), where pattern separation of complex odor information takes place (Doucette et al., 2007, 2011; Doucette and Restrepo, 2008; Gschwend et al., 2015; Gödde et al., 2016). We have recently shown that pattern separation of OB output neurons predicts behavioral performance of animals in an odor discrimination task (Gschwend et al., 2015; Gödde et al., 2016) and that exciting or inhibiting GABAergic interneurons in the granule cell layer can bidirectionally alter pattern separation and odor discrimination behavior (Gschwend et al., 2015). Nevertheless, it remains unknown whether and how pattern separation could evolve in a native OB network during odor discrimination learning. Moreover, there are two types of output neurons in the mammalian OB, mitral and tufted cells (MCs and TCs, respectively). They receive excitatory inputs from olfactory sensory neurons (OSNs) in common glomeruli (Mori et al., 1999; Shepherd et al., 2004) and in turn send output projections to distinct cortical and subcortical regions of the brain (Igarashi et al., 2012; Nagayama et al., 2010). Recent studies have started to shed light on the functional differences between MCs and TCs (Fukunaga et al., 2012; Igarashi et al., 2012; Kikuta et al., 2013; Nagayama et al., 2004), and yet little is known about the plasticity of the ensemble odor representations formed by the two populations in a behaviorally relevant context.

In this study, we chronically recorded the activity of MCs and TCs with two-photon imaging in awake head-fixed mice expressing a genetically encoded Ca2+ indicator. We investigated how the ensemble odor representation and pattern separation by MCs and TCs may change during (1) repetitive passive odor experience, where animals were simply exposed to odor stimulation...
over consecutive days and (2) odor discrimination learning, where animals were actively engaged in discrimination of two similar odors in a go/no-go task.

RESULTS

Odor Responses in MCs and TCs Recorded with Two-Photon Ca\textsuperscript{2+} Imaging

We first aimed at comparing odor-evoked response profiles in identified populations of OB projection neurons (see STAR Methods for the list of odorants used). Neuronal activity was monitored with the genetically encoded Ca\textsuperscript{2+} indicators GCaMP3/6s (Chen et al., 2013b; Tian et al., 2009) in awake head-restrained mice using two-photon laser-scanning microscopy (Figure 1A). Indicator expression was genetically restricted to mitral and tufted cells (MCs and TCs, respectively) by using a Pcdh21-CreER line (Nagai et al., 2005; Wachowiak et al., 2013; Yonekura and Yokoi, 2008) injected with adeno-associated viruses (AAVs) or crossed to a transgenic line, both of which express GCaMP in a Cre-dependent manner (see STAR Methods).

Taking advantage of the highly organized layer structure of the OB cortex, we then varied the depth of the focal plane in order to selectively image odor-evoked responses in either MC or TC populations (Figure 1B).

Although MCs and TCs are known to display complex firing patterns in awake mice, including increase and decrease of firing both during and after odor application (Cury and Uchida, 2010; Gschwend et al., 2015; Patterson et al., 2013; Shusterman et al., 2011), previous studies using longitudinal two-photon Ca\textsuperscript{2+} imaging in MCs and TCs mainly reported an increase in Ca\textsuperscript{2+} signals during odor application (Kato et al., 2012, 2013; Kato et al., 2017), http://dx.doi.org/10.1016/j.neuron.2017.02.006. These data guarantee that the odor-evoked inhibition is prevalent across different concentrations and that the concentration dependency is therefore not likely to be intrinsic to cells or odorants, but rather dependent on cell-odor combinations.

Concentration Dependency of Odor Responses in MCs and TCs

We next asked whether MCs and TCs could display a differential concentration dependency. The odor concentration tested was either higher (0.2) or lower (0.01) than or the same as that used for the rest of the study (0.05). For all response types (excitatory/inhibitory × ON/OFF), the peak amplitudes were significantly different across concentrations, with the highest concentration producing the largest amplitudes (Figures 2A and 2B). Overall, the proportions of responsive cell-odor pairs were not significantly different across concentrations (Figure 2C) or cell types (two-way repeated-measures ANOVA, ON Ex: p = 0.50; ON Inh: p = 0.87; OFF Ex: p = 0.32; OFF Inh: p = 0.02). MCs and TCs thus displayed similar concentration dependency. These data guarantee that the odor-evoked inhibition is prevalent across different concentrations and that the concentration used throughout the study (0.05) was unlikely to saturate the Ca\textsuperscript{2+} indicator.

Constant Reorganization of Ensemble Odor Representation following Passive Sensory Experience

A previous study, exclusively focusing on excitatory responses in MCs, showed that passive sensory experience (such as repetitive exposure of mice to odorants) could induce a pronounced weakening of response amplitude and sparsening of responsive cells (Kato et al., 2012). However, it remains unclear whether TCs and inhibitory responses could also undergo such plastic changes. The impact on inhibitory responses is particularly important since the reported plasticity was exclusively observed in the awake state and was attributed to an increase in GABAergic neuron excitability.

We repeated odor application (2 s for 11 trials) for 7 consecutive days. We first sorted the cell-odor pairs based on the response polarity on the first day. Consistent with and extending the previous study (Kato et al., 2012), we observed a weakening of response amplitude for both excitatory and inhibitory responses in MCs as well as TCs (Figures 3A–3C). However, the percentage of responsive cell-odor pairs remained stable across all days (Figure 3D, “Responsive”; note that all the p values are above 0.05 when a Bonferroni correction for multiple
Figure 1. Comparable Odor-Evoked Response Profiles in MCs and TCs
(A) Schema of the experimental paradigm.
(B) Schema of the OB circuitry and example images of TCs (~120 μm deep; green) and MCs (~220 μm deep; magenta) expressing GCaMP6s.
(C) Trial-averaged example traces (mean ± SEM) and pseudo-color heatmaps (bottom) of statistically significant responses evoked by 2 s odor application (black bars, gray boxes, and dotted lines). Cell-odor pairs were sorted by their relative peak amplitude and grouped according to their responses observed either during (ON) or after (OFF, 3 s post-odor) odor application. Excitatory (ΔF/F > 0) and inhibitory (ΔF/F < 0) responses are shown in red and blue, respectively. Data were acquired from 1,460 cell-odor pairs, 208 cells and 9 mice for MC; 1,081 cell-odor pairs, 163 cells and 7 mice for TC, respectively.
(D) Distribution of response profiles. The percentage of cell-odor pairs was calculated for each mouse (gray lines), and further averaged across mice (filled bars). Ex, excitatory; Inh, inhibitory; Sum, sum of excitatory and inhibitory responses.
(E) Response sign (ON and OFF responses were pooled). A given cell can either be solely excited (Ex only) or inhibited (Inh only) by all tested odorants, or be excited by some and inhibited by others (Both). The percentage of cells was averaged across odors for each mouse (gray lines) and further averaged across mice (filled bars).
(F) Response timing (excitatory and inhibitory responses were pooled). A given cell can be responsive either solely during (ON only) or after (OFF only) presentation of all tested odorants, or in ON period to some odorants and in OFF period to others (Both). Data shown as in (E).
(G and H) Percentage of cells for each response sign (G) and response timing (H) were averaged across animals (number of animals shown in white) and compared across odors (Kruskal-Wallis ANOVA). See STAR Methods for odor abbreviation.
(I) Cumulative probability distribution of peak amplitudes was compared between MCs and TCs (KS, Kolmogorov-Smirnov). The numbers of cell-odor pairs are shown for MCs and TCs, respectively. Data presented as mean ± SEM.
Figure 2. Concentration Dependency of Odor Responses in MC and TC Populations

(A) Example traces and pseudo-color heatmaps (bottom) of statistically significant responses evoked by 2 s odor application (black bars, gray boxes, and dotted lines) with different concentrations (cell-odor pairs were sorted by peak amplitudes at concentration 0.2). Data were acquired from 415 cell-odor pairs, 83 cells, and 3 mice for MC, and 340 cell-odor pairs, 68 cells, and 3 mice for TC. (B) Peak amplitude of odor responses in each cell-odor pairs for each concentration. Data from all pairs (gray lines) were averaged (colored bars) and compared across concentrations (Friedman ANOVA). The numbers of cell-odor pairs are shown. (C) Percentage of responsive cell-odor pairs for each concentration was averaged across mice and compared across dilution factors (Friedman ANOVA). Data presented as mean ± SEM.

Comparison is considered, indicating that new responsive pairs appeared after the first day. Interestingly, the response of initially responsive pairs also continued changing over days (Figure 3D, “Fate”): for instance, some excitatory pairs on day 1 remained excitatory, but others switched to inhibitory or sub-threshold, implying drastic reorganization of ensemble odor representation. Consistently, the decrease of amplitude of population responses appeared more modest when we averaged all the excitatory or inhibitory responses on each day without sorting by the response polarity on day 1 (Figure S2A). These data suggest that passive sensory experience does not necessarily cause a major sparsening of the odor response but rather induces a reorganization of the cell assemblies responding to the odorants.

To further examine the stability of ensemble odor representation, we performed a population vector analysis (Bathellier et al., 2008; Friedrich et al., 2004; Friedrich and Laurent, 2001; Gschwend et al., 2015; Mazor and Laurent, 2005) by combining all imaged neurons for each animal (Figure S2B). Pearson’s correlation coefficient was then computed between a pair of population vectors, which were constructed from responses to either (1) the same odors from a pair of different days (Figure 3E, “Within odors” comparison) or (2) a pair of different odors within the same days (Figure 3E, “Between odors” comparison). The correlation within the same odors, which indicates the stability of ensemble odor representation, markedly dropped across days (Figure 3E, “Within odors”), and this was the case no matter which day was used as a reference (Figure S2C). In contrast, the correlation between odors, which indicates the degree of pattern separation, was stable across days in both MCs and TCs (Figure 3E, “Between odors”). These results suggest that the ensemble representation of each odor in MCs and TCs underwent constant reorganization but that the ensemble representation still remained well separated between different odors.

What may be the impact of ensemble reorganization on odor discriminability during passive sensory experience? We addressed this question by performing odor classification based on a template-matching algorithm (Figure 4) (Bathellier et al., 2008; Gschwend et al., 2012; Mazor and Laurent, 2005; Stopfer et al., 2003). The reference templates could be selected either from the same day from which sample vectors were constructed, or from another day, the latter testing how much information the ensemble contains to classify odor responses on different days (see STAR Methods). In all cases, the classification curves were higher than chance both during the ON and OFF periods, though the accuracy was systematically higher during the ON period, as previously observed with electrophysiological recordings (Patterson et al., 2013). Consistent with the ensemble correlation analysis, the percentage of success for within-day odor classification remained stable across days (Figures 4A and 4B). Interestingly, the ensemble activity recorded on 1 day contained enough information to accurately predict the presented odorants on another day (Figures 4A and 4C). Moreover, successful classification was higher when the reference was taken from the later days than earlier days (Figure 4C): responses of day 7 could classify those of day 1 more reliably than the opposite despite the change in response amplitude and ensemble reorganization (Figures 4D and 4E).
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Figure 4. The Odor Representation Stabilizes after Passive Sensory Experience
(A) Traces of percentage successful odor classification over time. Responses of sample days were classified by those of different reference days (day 1 and day 7 for top and bottom panels, respectively). Data were averaged across mice (n = 9, data from TC and MO were combined since no difference could be observed between the two population of neurons) for each sample day. Black bars and gray boxes: 2 s odor application. Dotted lines: chance level (14%). (B) Successful classification within days. Responses were classified within the same days. Percentage of successful classification was averaged during odor application, normalized by the value of the day 1, and further averaged across mice. The classification remained stable across days (repeated-measures ANOVA). (C–E) Successful classification across days. Responses of a sample day were classified by those of a reference day. Percentage of successful classification was averaged during odor application for each sample day, normalized by that of a reference day, and further averaged across mice. (C) Pseudo-color heatmap with rows representing reference days and columns sample days. (D) Data with day 1 and 7 as reference. (E) Classification of day 7 responses by day 1 responses and that of day 1 responses with day 7 responses. Values from individual mice are shown with gray lines and the average across mice with red lines. Classification of day 1 responses by day 7 responses was better than the opposite (Wilcoxon’s sign-rank test).

Data presented as mean ± SEM.

In summary, our data suggest that, both in MCs and TCs, passive sensory experience reorganizes ensemble representation, while maintaining within-day odor discriminability and even increasing across-day odor predictability. In contrast to a previous report (Kato et al., 2012), this form of ensemble plasticity equally alters both excitatory and inhibitory responses without major sparsening (see also Discussion).

Improved Pattern Separation in MCs but Not in TCs upon Active Sensory Learning
When passive sensory experience alone can drastically modify the responses of both types of output neurons in the OB, what would be the consequences of active sensory learning? To address this question, we aimed to analyze responses of MCs and TCs while mice learned to perform a go/no-go odor discrimination task under head fixation (Abraham et al., 2012) (Figure 5A). A pair of binary mixtures evoking highly correlated activity in M/T cells in naive animals (Gschwend et al., 2015) was selected as odors to be discriminated. The performance of mice improved from near chance level (50% correct responses) on day 1 to well above the learning threshold (80% correct responses) on day 6, with no significant differences between mice imaged for MCs and those for TCs (Figure 5B). The learning pace of these animals appeared similar to that of the animals not expressing Ca2+ indicators in our previous study (Gschwend et al., 2015), making it unlikely that the expression of genetically

Figure 3. Similar Reorganization of Odor Representation in MCs and TCs after Passive Sensory Experience
(A) Ca2+ responses over 7 days of passive sensory experience. Traces averaged across all cells based on the peak polarity on day 1 and pseudo-color heatmaps (bottom) of all the responses evoked by 2 s odor application. Data were acquired from 700 cell-odor pairs, 100 cells, and 5 mice for MC; 665 cell-odor pairs, 95 cells, and 4 mice for TC, respectively. Cell-odor pairs were sorted by peak amplitudes during ON period on day 1. (B) Cumulative probability distribution of peak amplitudes was compared between different days. The numbers of cell-odor pairs for days 1 and 7 are shown in brackets. (C) Change of pairwise peak amplitude across days. The peak amplitude on day 1 was used to classify cell-odor pairs as excitatory (red) or inhibitory (blue). The response amplitudes showed a weakening across days (Friedman ANOVA: MC Ex n = 380, MC Inh n = 320, TC Ex n = 352, n = 313 cell-odor pairs). (D) The percentage of responsive cell-odor pairs and the fate of cell-odor pairs that were initially excitatory, inhibitory, and subthreshold on day 1. The percentage of responsive cell-odor pairs was averaged across mice and compared across days (Friedman ANOVA). (E) Correlation of ensemble responses. For each mouse, pair of population vectors were constructed from ΔF/F values of responses either to the same odors on a pair of different days (Within odors), or to a pair of different odors on the same days (Between odors). Pearson correlation coefficient (CC) was calculated between the two population vectors, averaged during 5 s after odor onset, then averaged across different odors (Within odors) or odor pairs (Between odors), and further averaged across mice.

Data presented as mean ± SEM.
A. Odor discrimination task

- Licking tube
- 2PLSM Odor application
- Odor discrimination task

B. Behavioral performance

- Mann-Whitney test
- KS test
- Chance level
- Correct response (%)
- Learning threshold

C. MC training vs TC training

- Ex
- Inh
- Cumulative probability
- Mean ± SEM
- Mann-Whitney test
- Ex
- Inh
- Cumulative probability

D. Mann-Whitney test

- Day 1 vs Day 6
- Mean ± SEM
- Ex
- Inh
- Cumulative probability

E. Ex
- Inh
- Cumulative probability

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encoded Ca\(^{2+}\) indicator in the OB output neurons affected the critical cellular plasticity involved in learning.

The responses of MCs and TCs between day 1 and day 6 were thus compared ("MC training" and "TC training"). As a control, we also passively exposed animals to the same pair of odors for the same number of trials and days ("MC passive"). As observed during passive sensory experience, when we selected the cells that responded during the first day, we observed a weakening of response amplitude for both excitatory and inhibitory responses regardless of cell type or context (Figures 5C–5E). Overall, excitatory responses to S– in MCs were most prominently reduced after learning, which was consistently observed for each individual mouse (Figure S3).

What happens to the ensemble activity during learning? The pseudo-color heatmaps of activity from all cells present the complex reorganization of odor responses during learning (Figures 6A–6C); some pairs changed the response sign, while others switched from responsive to non-responsive or vice versa (Figure S4). The percentage of responsive cells did not change significantly during learning (Figures 6D–6F; note that all the p values are above 0.05 when a Bonferroni correction for multiple comparison is considered). These data suggest that active sensory experience also induces the reorganization of responding cell assemblies rather than the sparsening of odor-evoked responses.

Could the reorganization of cell assemblies during active sensory learning improve pattern separation? To address this question, we quantified the correlation between the ensemble activity evoked by S+ and S– in the different cell types and/or contexts. The ensemble correlation between S+ and S– significantly decreased in MCs during training but neither in TCs during training nor in MCs during passive experience (Figures 7A–7D, "S+ versus S–", one way repeated ANOVA p = 4 \times 10^{-3}, 0.43 and 0.06 for MC training, TC training and MC passive, respectively). The baseline correlation remained close to zero across days in either group. The ensemble correlation in MCs gradually declined during training (Figure 7C, "S+ versus S–"); and reliably predicted the daily behavioral performance of mice (Figure 7B).

These data suggest that the pattern separation indeed evolves in the OB network in parallel with learning and that it was specific to MCs under the active learning context. On the other hand, the correlation between S+/S+ and that between S–/S– dropped significantly in all conditions though no significant difference was observed between groups (Figures 7C and 7D, "S+ versus S+", "S– versus S–"), indicating that the ensemble representation undergoes dynamic reorganization in a cell-type- and context-independent manner.

We addressed several factors that might have confounded our observations. First, the ensemble correlation could have been affected by breathing, which was previously shown to modify the inputs from OSNs (Verhagen et al., 2007). This scenario is unlikely, since the breathing frequency was not significantly different between groups (Figure 7E) despite a trend to slightly decrease over days (MC training: 4.23 ± 0.07 Hz versus 3.61 ± 0.14 Hz; TC training: 4.06 ± 0.19 Hz versus 3.64 ± 0.17 Hz; MC passive: 4.42 ± 0.19 Hz versus 3.68 ± 0.44 Hz; mean ± SEM, day 1 versus day 6, respectively). Moreover, the breathing frequency difference poorly predicted the ensemble correlation (Figure 7F). Second, smaller amplitude of responses in MCs compared to those in TCs might have resulted in lower correlation between S+ and S–. This is also unlikely to be the case, since (1) the ensemble correlation within the same mixtures in TCs was as low as that in MCs (Figures 7C and 7D, "S+ versus S+", "S– versus S–") and (2) the mean or the difference of population response amplitudes between S+ and S– could not fully account for the ensemble correlation (Figures S5A and S5B).

Finally, movements caused by licking might have resulted in lower ensemble correlation, as animals stopped licking to S– when they learned the task. This possibility is contradicted by the fact that (1) it was only in MCs that the ensemble correlation significantly decreased, while licking difference between S+ and S– was exist both in MCs and TCs after active learning, and (2) the MC ensemble correlation remained low even when the calculation was done with the data from "Miss" trials (failure to lick to S+) on the days when behavioral performance reached the learning threshold (Figure S5C). The latter evidence also argues against the possibility that MCs and TCs may be differentially sensitive to reward or arousal evoked by reward.

Taken together, MCs but not TCs displayed improved pattern separation after active sensory learning.

**A Network Model Predicts that Cortical Feedback Could Account for Context-Specific Pattern Separation in MCs**

Which circuit elements could be responsible for the context-dependent plasticity that we reported earlier? A recent report

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**Figure 5. Changes of Odor Response Amplitudes in MCs and TCs after Active Sensory Learning**

(A) A schema of the odor discrimination task. Head-fixed mice were trained to discriminate between two binary mixtures of odorants, one rewarded (S+) upon licking and the other unrewarded (S–). One second after a tone cue, odor was applied for two seconds during which licking was evaluated. For a correct response to S+, water reward was given at the end of the odor application.

(B) The odor discrimination performance. The mean across mice is plotted for each day (left). The performance was not significantly different between mice used for imaging MCs (n = 5 mice, magenta) and TCs (n = 5 mice, green). Data from individual mice are shown with circles, and the average across mice with bars.

(C) Changes of pairwise peak amplitude between day 1 and day 6 for each cell. The peak amplitude on day 1 was used to classify cells as excitatory (red) or inhibitory (blue). ΔF/F values from individual cells from all mice were pooled and averaged. The numbers of cells are shown in brackets. Data from individual cells are presented with gray (amplitude decrease or change of polarity) or gray green (amplitude increase) lines, and the average across cells with solid lines.

(D) Cumulative probability distribution of peak amplitudes was compared between day 1 (black) and day 6 (yellow). The numbers of cell-odor pairs for day 1 and 6 are shown in brackets.

Data presented as mean ± SEM.
Figure 6. Changes of Ensemble Activity during Active Sensory Learning

(A–C) Pseudo-color heatmaps of odor responses from all cells for MC training (A), TC training (B), and MC passive experience (C). Cell-odor pairs were sorted by peak amplitudes during S+ presentation on day 1.

(D–F) Percentage of responsive cells during odor application for MC training (D), TC training (E), and MC passive experience (F). Data were averaged across mice and compared across days (Friedman ANOVA; n = 5, 5, and 3 mice for each condition). Data presented as mean ± SEM.
Bayesian inference (see STAR Methods). Upon odor stimulation of a particular odor in a mixture through approximate modifying the feedforward drive to cortex, a process that repeats itself until most of the MC activity has been properly predicted by cortical activity.

In the naive state, the circuit has a fixed repertoire of odor response templates, with a single cortical cell assigned to represent a particular odor with specific inputs from MCs and corresponding feedback weights projecting to granule cells. Initially the circuit model responds to a new odorant by interpreting it as a sparse mixture of the existing templates. For this purpose, we modified an existing computational model that successfully solves “demixing” problems (Grabska-Barwińska et al., 2017), i.e., identifies the presence of a particular odor in a mixture through approximate Bayesian inference (see STAR Methods). Upon odor stimulation, excitatory inputs are relayed from OSNs to MCs and from MCs to cortical cells. Cortical cells then in turn send a feedback signal to the bulb corresponding to the predicted MC responses given the mixture of odors identified in cortex (Figure 8A). This feedback signal inhibits MC responses, thus modifying the feedforward drive to cortex, a process that repeats itself until most of the MC activity has been properly predicted by cortical activity.

We thus examined the effect of cortical feedback on context-dependent improvement of pattern separation in MCs but not in TCs (Otazu et al., 2015). We demonstrated that cortical feedback on the OB could contribute to the pattern separation in MCs but not in TCs. For this purpose, we modified an existing computational model that successfully solves “demixing” problems (Grabska-Barwińska et al., 2017), i.e., identifies the presence of a particular odor in a mixture through approximate Bayesian inference (see STAR Methods). Upon odor stimulation, excitatory inputs are relayed from OSNs to MCs and from MCs to cortical cells. Cortical cells then in turn send a feedback signal to the bulb corresponding to the predicted MC responses given the mixture of odors identified in cortex (Figure 8A). This feedback signal inhibits MC responses, thus modifying the feedforward drive to cortex, a process that repeats itself until most of the MC activity has been properly predicted by cortical activity.

In the naive state, the circuit has a fixed repertoire of odor response templates, with a single cortical cell assigned to represent a particular odor with specific inputs from MCs and corresponding feedback weights projecting to granule cells. Initially the circuit model responds to a new odorant by interpreting it as a sparse mixture of the existing templates. Then, following repeated exposure to a new odor, we assume that the passive sensory experience leads one to repeat itself until most of the MC activity has been properly predicted by cortical activity.
frequent occurrence), thereby increasing the feedback inhibition to the MCs.

This configuration led to better identification of the novel odors as demonstrated with linear classification of cortical activation patterns (Figure S6A). However, in the case of active learning of closely related odorants, simply having two new units for S+ and S− is not sufficient to disambiguate the two odorants (Figure S6B). It is also important that S+, which is associated with a reward, be identified as quickly as possible. For the active learning, we thus implemented the model with preemptive activation of cortical cells representing S−, leading to specific inhibition of S− responses in MCs so as to enhance the rapid detection of S+ (see STAR Methods). This in turn resulted in differential weakening of the MC response to S+ versus S− after active learning, with the response to S+ remaining stronger compared to the response to S−.

The model thus reproduced our major experimental findings: (1) odor responses of MCs are weakened both after passive sensory experience and active learning; however, this weakening after active learning is reduced for S+ compared to S−, reflecting the different task-specific cortical processing of the two odors (Figures 8B–8D); (2) ensemble correlation between S+ and S− after passive sensory experience or active learning, normalized by the values in the naive state. Pex, Passive excitatory; Pin, Passive inhibitory; Aex, Active excitatory; Ain, Active inhibitory.

Comparison with the Previous Two-Photon Ca2+ Imaging Studies in the OB

Surprisingly, odor-evoked inhibition has been overlooked in most studies using two-photon Ca2+ imaging in the OB (Kato et al., 2012, 2013; Otazu et al., 2015) except for a very recent report (Economo et al., 2016), although cellular imaging therein was performed exclusively under anesthesia. Our unbiased
sampling showed relatively balanced prevalence of excitation and inhibition in different concentrations of odorants (Figures 1 and 2), which is consistent with electrophysiological recordings in awake animals (Cury and Uchida, 2010; Gschwend et al., 2015; Kollo et al., 2014; Shusterman et al., 2011). Our work thus provides solid evidence supporting the presence of odor-evoked inhibition at a cellular level under awake condition. The discrepancy might be due, at least in part, to the less sensitive Ca$^{2+}$ indicator and the use of different criteria for selecting significant responses in the previous studies (Figure S7).

It was previously reported that MCs show a drastic weakening of response amplitude as well as sparsening of responsive cells during passive sensory experience (Kato et al., 2012). Regarding the weakening, we not only replicated but also extended these results to TCs and inhibitory responses, which is far from trivial for considering the circuit mechanisms underlying this phenomenon (see the next section). In contrast, we did not find evidence supporting a robust sparsening; instead, our results show that there was a major reorganization of the cell assemblies responding to the odorants, with initially non-responsive cells being recruited during the passive sensory experience. Again, this discrepancy may be due, at least in part, to the less sensitive Ca$^{2+}$ indicator and the use of different criteria for selecting significant responses in the previous studies.

We finally addressed the impact of associative learning on ensemble representations. The change in MC representation is consistent with a recent report (Chu et al., 2016), though we observed neither a strong sparsening of representation during learning (Figure 6) nor improved pattern separation during passive sensory experience (Figure 7). Furthermore, we showed that the ensemble pattern separation in MCs but not in TCs improves simultaneously as animals learn to discriminate perceptually similar odors (Figure 7).

Comparison with Previous Studies Using Other Techniques

We characterized fluorescence changes in response to odor exposure in individual neurons with Ca$^{2+}$ imaging. Although the speed of Ca$^{2+}$ changes is not fast enough to follow the modulation of firing within a single breath, we could still successfully record both increase (excitatory) and decrease (inhibitory) in fluorescence during (ON response) and after (OFF response) odor application in both cell types (Figures 1 and S1). This is presumably owing to the fact that Ca$^{2+}$ changes represent firing rate changes accumulated over time (Yaksi and Friedrich, 2006).

We found that a majority of cells responded with excitation or inhibition depending on applied odors but that a smaller proportion of cells responded to all tested odors exclusively with excitation or inhibition (Figure 1E). These might correspond to spontaneously silent and active cells that mainly show excitatory and inhibitory odor responses, respectively (Kollo et al., 2014).

MCs and TCs showed similar concentration dependency in our hands, which is somewhat in contrast to the previous reports suggesting that TCs may be more sensitive to lower concentrations of odors than MCs (Gire et al., 2012; Igarashi et al., 2012; Kikuta et al., 2013). The concentrations we tested may be limited to a higher range, although direct comparison is hampered by differences in preparations and/or techniques (i.e., awake versus anesthetized, in vivo versus in vitro, Ca$^{2+}$ imaging versus electrophysiology).

It was previously reported by electrophysiological recording that the firing rate of the OB output neurons temporarily changes during active sensory learning (Doucette and Restrepo, 2008). Although the ensemble correlation analysis was not performed, this could translate as a weak and transient improvement of pattern separation in our setting, which would be in contrast to the gradual development of significant pattern separation that we observed in MCs. A potential source of this discrepancy could be the difficulty of the task: the sets of odorants these authors used for the task were easier to discriminate than ours and therefore might not necessarily involve improvement of pattern separation upon learning (Chu et al., 2016; Gödde et al., 2016; Gschwend et al., 2015). Moreover, with multi-unit recording, they could neither distinguish MCs and TCs nor track the same cell ensembles across multiple days, demonstrating the advantage of the chronic imaging technique we employed in the current study.

Circuit Mechanisms Underlying Different Forms of Plasticity

The first type of plasticity, constant reorganization of ensemble odor representation, was observed both in MCs and in TCs regardless of behavioral context (either in passive experience or in active learning). As noted above, we found a general weakening of both excitatory and inhibitory responses, as well as continuous reorganization of ensemble odor representation on a daily basis. Interestingly, this was similarly observed in both populations of output neurons. Since synaptic inputs from OSNs were reported to remain relatively constant during repeated passive sensory experience (Kato et al., 2012), the circuits within and/or beyond the OB should be responsible for this form of plasticity. Since both the excitation and inhibition were subject to weakening, a general gain control of the overall OB activity might partly account for these reorganizations, although it might not easily explain the appearance of newly responding neurons during the course of the repetitive daily exposure or the fact that the response to S+ is higher than S− only after active learning. The model presented here suggests that cortical feedback on inhibitory neurons might contribute to the cell-type- and context-independent reorganization. Anyhow, additional work will be necessary to study more precisely the circuitry involved in such plasticity.

In stark contrast, the second type of plasticity, improvement of pattern separation, was much more pronounced in MCs than in TCs and only observed during active learning. This is again likely ascribed to the circuit within and/or beyond the OB, since input patterns from OSNs evoked by closely related mixtures were reported to be highly correlated between S+ and S−—even after odor discrimination training (Abraham et al., 2004). The feedback inputs from the piriform cortex might be an attractive candidate as demonstrated by our circuit model (Grabska-Barwińska et al., 2017). Indeed, it was recently shown that in naïve mice the cortical feedback enhances the pattern separation of MC but not TC assemblies through yet-to-be identified inhibitory circuits (Otazu et al., 2015). In this scenario, OB interneurons in the granule cell layer (GCL) would be a key mediator, since (1) they
are the main target of the feedback axons (Otazu et al., 2015), and (2) our recent work showed that manipulating their activity bidirectionally affected both the pattern separation of output neurons and the odor discrimination performance of animals (Gschwend et al., 2015). Intriguingly, granule cells located at different depths of the GCL may form distinct connection with MCs and TCs (Mori et al., 1983; Orona et al., 1983, 1984) and have distinct physiological properties (Geramita et al., 2016). They hence might contribute to the difference in the plasticity between MCs and TCs. Additionally, all neuromodulatory inputs to the OB were shown to affect odor discrimination behavior (noradrenaline, Doucette et al., 2007; acetylcholine, Fletcher and Wilson, 2002; Linster et al., 2001; serotonin, Marchetti et al., 2000), although the task configurations varied across these studies and were different from ours. It will require extensive future investigations to evaluate whether these neuromodulations are indeed relevant in our setting, and how they might differentially affect the ensemble plasticity of MCs and TCs.

Functional Implication of Flexible Sensory Representation

The odor classification analysis during passive sensory experience showed that the ensemble responses on later days could predict the odorants applied on earlier days more accurately than the opposite (Figure 4). This might imply that the ensemble representation converges to a certain fixed state after the constant dynamic reorganization.

It is interesting to note that the pattern separation remained relatively stable during passive sensory experience despite the constant reorganization of sensory representation. This is reminiscent of highly stable ensemble representation despite the dynamic alterations of single-cell activity, reported for L2/3 neurons in the primary motor cortex (Huber et al., 2012) and for hippocampal place cells (Ziv et al., 2013). How could the flexible OB representations be read out by the target brain regions? In our model, this question is moot. The reorganization of the representation in the OB is precisely the reflection of the fact that the cortex has learned a specific representation of the new odors in the cortical layer, i.e., it has learned how to read out the OB representation. Alternatively, the repeated sensory experience could continuously reorganize the central odor representations as well, resulting in altered significance of the experienced odors. Chronic recording of odor responses in the higher olfactory areas would help address this question.

Cell-Type-Specific Improvement in Pattern Separation: Multiplexed Sensory Output Channels

In olfaction, it has been long speculated that TCs might contribute to odor detection, while MCs might facilitate odor discrimination, based on their spontaneous and odor-evoked activity timing (TCs early versus MCs late) (Fukunaga et al., 2012; Nagayama et al., 2010). To the best of our knowledge, our results provide the first direct evidence to this hypothesis, by showing that TCs and MCs display remarkable differences during active learning but not during passive experience. It is tempting to speculate that the OB circuitry is implemented with complimentary multiplexed output channels: TCs might ensure more reliable odor detection and/or maintain the information about odor similarity, while MCs might contribute more to pattern separation (Figure S8). In the future, it would be interesting to assess the behavioral consequences of separate manipulation of MCs and TCs, although there are no genetic tools currently available to target these two populations specifically and independently. It would be also important to examine the functional difference of the cortical areas and subregional compartments that are differentially innervated by MCs and TCs (Igarashi et al., 2012).

In the primary somatosensory cortex, layer 2/3 pyramidal cells that project to different areas form non-overlapping subpopulations, namely those projecting to the primary motor cortex (M1P) and those to the secondary somatosensory cortex (S2P). Recently, it was shown that M1P and S2P display distinct plasticity following active tactile learning (Chen et al., 2013a, 2015). Segregated output channels implemented with differential plasticity may therefore be a general and fundamental feature of early sensory circuits, expanding their capacity to transfer non-redundant sensory information to distinct downstream regions.

STAR Methods

Detailed methods are provided in the online version of this paper and include the following:

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Supplemental Information includes eight figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2017.02.006.

**AUTHOR CONTRIBUTIONS**

Y.Y., K.B., I.R., and A.C. carried out the study conceptualization and experimental design. Y.Y. and K.B. acquired and analyzed data. Y.Y. wrote the custom analysis scripts on MATLAB. K.B. built the two-photon microscope. T.J.M. and A.P. contributed computational models. Y.Y., T.J.M., and A.C. wrote the manuscript with comments from all the other authors.

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REFERENCES


STAR★METHODS

KEY RESOURCES TABLE

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CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for reagents may be directed to, and will be fulfilled by the corresponding author, Dr. Alan Carleton (alan.carleton@unige.ch).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All animal protocols were in accordance with the Swiss Federal Act on Animal Protection and the Swiss Animal Protection Ordinance, and were approved by the University of Geneva and Geneva state ethics committees (authorization numbers: 1007/3387/2, 1007/3758/2 and GE/156/14). Experiments were performed on 2-6 month-old male mice of the following genotype: Pcdh21-CreER hemizygous transgenic mice (Yonekura and Yokoi, 2008), or Pcdh21-CreER hemizygous transgenic mice crossed with Ai38 R26LSL-GCaMP3 homozygous transgenic mice (JAX no 014538; Zariwala et al., 2012). Mice were housed in groups of...
2–5 in a temperature- and humidity-controlled animal facility (12-h light/dark cycles). All animals were naive to procedures at the beginning of the experiment. Animals were randomly assigned to the various experimental groups.

METHOD DETAILS

Animal preparation
Prior to surgery, animals were anesthetized with an intraperitoneal injection of a mixture containing midazolam (8 mg/kg), medetomidine (0.6 mg/kg) and fentanyl (0.02 mg/kg). Dexamethasone (2 mg/kg) was intramuscularly injected to prevent brain swelling. Carprofen (5 mg/kg) was intraperitoneally injected for analgesia. All mice were allowed to recover for > 7 days after surgery and then habituated to be head restrained > 15 min/day for > 5 days. All experiments were done during daytime. Experiments were terminated when the clarity of the cranial window was not maintained (mostly due to bone regrowth).

Expression of genetically encoded Ca²⁺ indicator
Pcdh21-CreER hemizygous transgenic mice (Yonekura and Yokoi, 2008) were either 1) injected with Cre-dependent recombinant adeno-associated viruses expressing GCaMP3 (Tian et al., 2009) or GCaMP6s (Chen et al., 2013b); AAV9.Syn.Flex.GCaMP6s.WPRE.SV40 or AAV9.Syn.Flex.GCaMP3.WPRE.SV40, University of Pennsylvania Vector Core) at least 3 weeks prior to imaging, or 2) crossed with Ai38 R26LSL-GCaMP3 homozygous transgenic mice (JAX no 014538; Zariwala et al., 2012) (Figure 1a). Only data acquired with GCaMP6s were used for all the figures except Figure S7. Virus solutions (0.2-0.5 μl) were injected into each hemi-bulb with glass capillaries (tip diameter ~30 μm) at 250-400 μm below the pial surface. Tamoxifen (50-100 mg/kg) was intraperitoneally injected for 3 consecutive days to induce Cre-mediated recombination, typically one week after virus injection.

Two-photon Ca²⁺ imaging
Imaging was performed with a custom-built two-photon microscope controlled by ScanImage 3.7 (http://scanimage.vidriotechnologies.com). Either 20 × (NA0.95, Olympus) or 16 × (NA0.8, Nikon) objective lens was used. Ti-Sapphire laser (Chameleon ultra II, Coherent) was tuned to 910 nm. The fields of view corresponding to ~340 × 340 μm were imaged at a spatial resolution of 512 × 128 pixels and at a frame rate of 7.81 Hz. Separation of MCs and TCs was based on imaging depth; TCs were imaged in a plane below the glomeruli where cell bodies are embedded within dense neuropil, while MCs were imaged in a deeper plane where cell bodies are clustered in sparser neuropil. This could have led to inclusion of some deep tufted cells in the MC population.

Odor application
Saturated odorant vapor was diluted 20 times (except for the data in Figure 2) with air, and applied for 2 s at a flow rate of 400 sccm with a custom-made olfactometer. Odor application was synchronized to the beginning of inhalation, which was recorded (recorded at 10kHz) with an air pressure sensor placed in front of the other nostril. Odorants used include 3-Hexanone (3H), amyl acetate (AA), butyric acid (BA), (−)-Carvone (C−), ethyl butyrate (EB), ethyl valerate (EV), hexanal (HX), methyl benzoate (MB), methyl valerate (MV), and valeric acid (VA).

Odor discrimination task
Head-fixed animals were trained to discriminate 2 different binary mixtures of odorants in a lick/no-lick paradigm, as previously described (Abraham et al., 2012). Briefly, animals under water restriction regime (total of ~1 ml/day for each animal) were subjected to a pre-training protocol followed by a training protocol. The first stage of pre-training protocol aimed to provoke licking of a metal tube. 3 s after a tone (200 ms, 5000Hz) offset, a water drop (2 μl) was unconditionally delivered to animals via a metal tube for ~20 trials. Licking was detected as the electrical contact of the tube and the tongue of animals, and sampled at 500 Hz. In the second stage of pre-training protocol, 1 s after the tone offset, an odorant (−)-Carvone was presented for 2 s, and a water reward was delivered only if animals displayed successful licking, defined by the total duration of licking: odor period was divided into 4 epochs of 500 ms, and the cumulative duration of licking had to be above threshold in more than 1 epoch, which was gradually increased from 40 ms up to 160 ms (40 ms: 30 trials, 80 ms: 30 trials, 120 ms: 30 trials, 160 ms: 100 trials). If animals displayed licking during the pre-odor period (1 s immediately before odor onset), an alternative threshold was used: cumulative licking duration per epoch during the odor period had to be longer than that during the pre-odor period, with the ratio gradually increasing from 100% up to 200% (100%: 30 trials, 125%: 30 trials, 150%: 30 trials, 175%: 50 trials, 200%: 50 trials). Most animals completed the pre-training protocol within 3 days.
Animals were then subjected to a training protocol, where they were presented with two binary mixtures of odorants, one being rewarded (S+; AA60EB40) and the other being un-rewarded (S−; AA40EB60). The criteria for correct responses to S+ (‘hits’) were the same as those at the last step of pre-training: successful licking (cumulative licking duration of > 160 ms) in 2 or more epochs during the odor period, or > 200% of cumulative licking duration during the entire odor period compared to the pre-odor period, if any. The criteria for correct responses to S− (‘correct rejection’) were less than 2 epochs with cumulative licking duration of < 80 ms, or < 25% of cumulative licking duration during the entire odor period compared to that during the pre-odor period. No punishment was given to the mice for incorrect trials. Odorants were presented in a pseudo-randomized manner, where the same odor was repeatedly presented in no more than 2 consecutive trials, and the number for each odor was balanced within a block of 10 trials. Inter-trial interval was 20 s, and the number of trials was typically 150 each day per animal.

Computational models
The details of the model are described in our previous work (Grabska-Barwińska et al., 2017). Briefly, our model circuitry consists of four layers and five groups of variables: ORNs, MCs, cortical cells, and two compartments for each cell in the granule cell layer, with firing rates $r_i$, $m_i$, $c_i$, $g_i$ and $g_k$ respectively. The following equations govern the firing rates of the different cells and thus the dynamics of the system:

$$\tau_c \frac{dc_i}{dt} = \beta_j a_0 - c_j + \beta_j F_j(c_j) \sum_i \gamma_i^{-1} m_i^2 w_{ij},$$

$$\tau_m \frac{dm_i}{dt} = -m_i^2 v_0 + \gamma_i r_i - m_i \sum_k w_{ik}^m g_k,$$

$$\tau_g \frac{dg_k}{dt} = -g_k + g_k w_{ik}^m m_i,$$

$$\tau_v \frac{dg_k}{dt} = -g_k + \sum_i A_{ik} F_j(c_i),$$

where

$$F_j(c_i) = \psi c_i / (c_i + \beta_j),$$

with $\psi$ the digamma function,

$$\beta_j = \frac{\beta_0}{1 + \beta_0 \sum_i w_{ij}},$$

and

$$w_{ij} = \sum_k w_{ik}^m w_{kj}^m A_{ik}.$$

Here $w_{ij}$ is the weight corresponding to the activation of the $i$-th ORN by odor and $v_0$ is the background firing rate for ORN $i$. $w_{ik}^m$, $A_{ik}$ and $A_{ik}$ are the granule cell to mitral cell, mitral cell to granule cell, and piriform cortex to granule cell weights, respectively. Each mitral cell is associated with three primary granule cells, with reciprocal connections. Furthermore, each granule cell has three secondary mitral cells on either side, with the probability of a reciprocal connection being 0.5. Feedback from a cortical cell goes to either all three or none of a group of granule cells, with the probability of a connection being 0.2.

Further $\tau_i$ is the time constant for cell type $i$, $(\alpha_0, \beta_0)$ are parameters used in the variational approximation for Bayesian inference, and $\gamma_i$ is an arbitrary positive constant. The values of all parameters used are listed in Table S1.

The equation governing the cortical dynamics can be simplified through a change of variables $a_i = c_i / \beta_i$ to give

$$\tau_c \frac{da_i}{dt} = a_0 - a_i + \beta_i^2 \psi (a_i) \sum_i \gamma_i^{-1} m_i^2 w_{ij},$$

The network is comprised of 640 cortical cells (each representing one of 640 different odors), 160 MCs and ORNs, and 480 granule cells.

To obtain the calcium signals from the MC firing rates, the firing rate of each cell (represented by the $a_i$ value) was convolved with a negative exponential with a time constant of 3 s. This convolved signal was then added to a baseline Ca$^{2+}$ value (common to all cells), and normalized by its pre-stimulus average value cell-by-cell.

The new odors, S+ and S−, were chosen to be dissimilar to the existing repertoire of odors, but similar enough to each other to make distinguishing them a challenging task. In order to achieve this, first two base odors were chosen whose ORN activation vector had a small dot product with those of the existing odors. These base odors were then mixed in a 5/6-1/6 and 1/6-5/6 ratio to obtain S+ and S−.
Odors were presented to the model after a 9 s prestimulus period, for 2 s. Breathing cycles were modeled with the help of symmetric beta distributions with both coefficients at 1.2, with a frequency of 4Hz, such that during each 500-ms period the odor concentration moved from 0 to its peak value and back.

The passive exposure to S+ and S− results in twofold changes to the model circuitry.

First, cortical cells are introduced both for S+ and S−, replacing two of the previous odors (or, alternatively, augmenting the number of cortical cells by two—these two different methods yielded essentially identical results).

Second, the priors (the expectations of the animal about the presence and concentration of the odor) are adjusted to reflect the high relative frequency of these odors. We found that the network’s behavior suited the task best when this adjustment was implemented directly in the equation controlling the cortical dynamics, by increasing the prior-related term $\beta_j$ multiplying the feedforward activation from the mitral cell layer, so that the equation governing the cortical cells becomes

$$\frac{da_j}{dt} = a_0 - a_j + \beta_{\text{change}} F_j(c_j) \sum_i \gamma_i^{-1} m_i^2 w_{ij},$$

This change can also be interpreted as a uniform strengthening of these projection weights, since multiplying $\beta_j$ or the vector $w_{ij}$ by a scalar are equivalent. $\beta_{\text{change}}$ was set to 2.5.

To quantify the effect of these changes on the quality of the cortical code, we ran a four-way linear classification task using the average cortical activity during the 2 s odor presentation. On each trial, either S+, S−, neither, or both odors were present, as well as randomly chosen odors, such that the total number of odors in the mixture is 5. Classification performance was evaluated on 2000 trials (500 in each condition) using linear discriminant analysis with 10-fold cross-validation. Figure S6A shows the improvement in classification performance following passive learning.

The active learning task requires the animal to quickly distinguish whether S+ is present (in which case a licking response is required), or whether it is absent (in which case no response should be given). Given the task set-up, this determination should already be established during the first 500 ms of the trial (as responding is evaluated separately during the four equal parts of the 2 s trial duration). We therefore looked for a modification of the cortical code that would quickly and unambiguously enable such a readout from the cortical cell corresponding to S+, given varying odor concentrations/ORN response magnitude. As S+ and S− are similar odors, S− presentation can also induce S+ cell activation under the model configuration we introduced for passive learning, leading to an erroneous readout and performance error. While using a single cell to represent the presence of particular odors is an unrealistic simplification in the model, it serves as a proxy for a readout from a distributed cortical representation, where the animal’s behavior would presumably be driven by such an S+ signal. One way to overcome the problem of the S− odor activation the S+ cortical cell is to preemptively activate the S− cortical cell before odor presentation, to subtract away the ORN activity corresponding to the S− template, facilitating the detection of S+. We therefore modeled active learning as implementing such a preemptive activation mechanism (in addition to learning the two odors as in the passive exposure case). S− was therefore activated 40 ms before odor-evoked ORN activity began. The imposed activation acted as a minimum level of activity, above which the firing rate was allowed to rise as dictated by the network dynamics. This minimum activity threshold was set to decay linearly to 0 during the 2 s of odor presentation, from an initial firing rate of 18.57 (or $a_{S−} = 1000$).

These changes ensured that the firing of the S+ cortical cell remained low even at very high odor concentrations/ORN activity when S− was presented, while growing monotonically with concentration when S+ was presented. This enabled a perfect readout of the presence of S+ over a wide range of concentrations within the first 500 ms, by setting the appropriate decoding threshold. Even at very low concentrations, activity in the cortical cell rose above this threshold within the second 500-ms interval.

In contrast, under the passive exposure setup, activation of the S+ cortical cell when high concentration of S− is presented rose above the level of activity on presentation of S+ at low concentrations. This is shown on Figure S6B with a low concentration in the model of 1.75 (where the cortical cell started responding significantly in the active condition) and a high of 14.

For fitting the experimental data, $\beta_{\text{change}}$ reflecting the change in prior was modified so that the increase in the prior for S+ was smaller than for that in S−. This could correspond to an objective where the animal requires more to establish the presence of S+ and engage in the appropriate licking behavior.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Data analysis**

Image analysis was performed with custom-written scripts in ImageJ and MATLAB (MathWorks). Image frames were corrected for in-focal (XY) plane brain motion using cross-correlation based on rigid body translation (Dubbs et al., 2016), and spatially smoothed by a 3 × 3 median filter. The corrected stacks were inspected for out-of-focal (Z) plane brain motion with the following algorithm: correlation coefficients of fluorescent intensities from all pixels were calculated between a given frame and a reference image (average intensity projection of the most stable stack of the day), z-scored, and the frame was discarded from further analysis if the z-scored correlation coefficient was less than −2. Regions of interest (ROIs) were manually drawn over cell bodies, and average fluorescence intensity within each ROI was extracted. We calculated $dF/F$, or the change in fluorescence relative to baseline (4 s prior to odor application) divided by the mean of fluorescence during baseline.
A response was considered significant when the maximum of absolute $\Delta F/F$ values exceed twice the standard deviation of $\Delta F/F$ values during baseline. ON responses include any that were significant during odor application, while OFF responses include those that were significant only after cessation of odor application.

Ensemble correlation was calculated as described in Figure S2B. To calculate within-odor correlation (Figure 3E, ‘Within odors’; Figure S2C; Figures 7C, 7D, and 7F, ‘S+ versus S+’ and ‘S− versus S−’) with the reference and sample taken from the same day, the trials were randomly separated into two groups to generate two trial-averaged $\Delta F/F$ traces. The correlation coefficient was calculated between the two traces for each random sampling, and averaged across repetitions (maximum 1,000).

Odor classification was performed with a template-matching algorithm as previously described (Bathellier et al., 2008; Gschwend et al., 2012; Mazor and Laurent, 2005; Stopfer et al., 2003). Briefly, a sample population vector (containing $\Delta F/F$ values of a given time point from all the imaged cells in a given animal) was constructed from a given trial (odor X). A reference population vector was constructed from trial-average traces of each odor; for odor X, the sample trial was excluded from averaging. The euclidean distance was calculated between the sample vector and each reference vector, and trial was considered successfully classified when the distance between the sample and odor X reference was the smallest. The percentage of successful classification was calculated as the number of successfully classified trials divided by the total number of trials. Templates were taken from the same day as test trials (for classification within days; Figure 4B) or from another day (for classification across days; Figures 4C–4E).

Breathing signals (exhalation positive, inhalation negative) were automatically analyzed. Traces were first band-pass filtered (second-order Butterworth filter, 1-25Hz; (Wesson et al., 2008), and a point was defined as the beginning of inhalation and used for quantifying the breathing frequency if it 1) crosses zero, 2) has a negative slope, and 3) resides within 120 ms before an inhalation peak. The frequency was calculated as the mean during 5 s after odor onset.

For the passive exposure to simple odors, the first trial of each odor application was excluded from further analysis to eliminate the effect of fast sniffing (Kato et al., 2012; Wesson et al., 2008). For the odor discrimination task, the first 10 trials of each day were also excluded from further analysis since the behavioral performance tended to be unstable.

Statistics
All statistical analyses were performed with MATLAB or Prism. We used parametric and non-parametric ANOVA, Kolmogorov-Smirnov test, and Mann-Whitney test. All tests were two-sided. Shapiro-Wilk test was used to assess normality of the data. The error bars represent standard error of the mean. No statistical methods were used to predetermine sample sizes, but our sample sizes were similar to those reported in previous publications (Abraham et al., 2014; Gschwend et al., 2015; Tatti et al., 2014; Vincis et al., 2012). None of the experiments were blind to the genotype or cell type, although the data from different groups were processed by the same analytical codes to avoid any bias.

DATA AND SOFTWARE AVAILABILITY
The data supporting the findings of this study and the codes used to analyze the data are available upon reasonable request.
Supplemental Information

Context- and Output Layer-Dependent

Long-Term Ensemble Plasticity in a Sensory Circuit

Yoshiyuki Yamada, Khaleel Bhaukaurlally, Tamás J. Madarász, Alexandre Pouget, Ivan Rodriguez, and Alan Carleton
SUPPLEMENTAL INFORMATION

Context- and output layer-dependent long-term ensemble plasticity in a sensory circuit

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SUPPLEMENTAL INFORMATION

Figure S1.
Heat maps of all cell-odor pairs and histograms of half maximum time points (related to Figure 1).

Figure S2.
Net peak amplitude, schema of population vector analysis and within-odor correlation computed with different reference days (related to Figure 3).

Figure S3.
Changes of response amplitude after active learning and passive experience for individual mice (related to Figure 5).

Figure S4.
The fate of cell-odor pairs during active sensory learning and passive sensory experience (related to Figure 6).

Figure S5.
Ensemble correlation vs. population response amplitudes and ensemble correlation in error trials (related to Figure 7).

Figure S6.
Model performance after passive experience and active learning (related to Figure 8).

Figure S7.
Data with different Ca^{2+} indicators and distribution of response profiles with different criteria (related to Figure 1 and Discussion).

Figure S8.
Schema representing the implications of the findings (related to Discussion).

Table S1.
Values of all parameters used for the model (related to Figure 8 and STAR Methods).
Figure S1

Figure S1. Heat maps of all cell-odor pairs and histograms of half maximum time points (related to Figure 1).

(A) Pseudo-color heat maps of $\Delta F/F$ values from all cell-odor pairs (including those that are not statistically significant) evoked by 2-s odor application (dotted lines). Cell-odor pairs were sorted by their relative peak amplitude during odor application. Data shown as in Figure 1C.

(B) Histogram of half maximum time points (shown in frames) calculated for ON and OFF responses (black and white boxes respectively). Odor period is shown with dotted lines. The two distributions were statistically different (KS test: Kolmogorov-Smirnov test).
Figure S2

(A) Changes of net peak amplitude (continuous lines) and pairwise peak amplitude (dotted lines) across days. For the former, the peak amplitude of each day was used to classify cell-odor pairs as excitatory or inhibitory. For the latter, the peak amplitude of day 1 was used (replicated from Figure 3C). They were statistically different (Mann-Whitney test; * at least $P < 6 \times 10^{-4}$ after Bonferroni correction for multiple comparison).

(B) Schema of population vector analysis. Trial-averaged responses of n cells in two different conditions were sliced at time point x to construct population vectors. The resultant vectors have n values of $\Delta F/F$. The correlation coefficient between the two vectors was calculated, and this was repeated for all time points.

(C) Within-odor ensemble correlation computed with different days set as reference. The panel ‘day1’ is replicated from Figure 3E ‘Within odors’. The correlation was statistically different across days regardless of the reference, but not between cell types (two-way repeated measures ANOVA).

Data presented as mean ± SEM.
Figure S3. Changes of response amplitude after active learning and passive experience for individual mice (related to Figure 5).
Pair-wise peak amplitude changes between day 1 and day 6 for each cell, plotted for each mouse. Data are presented as Figure 5D (mean ± SEM).
Figure S4

Figure S4. The fate of cell-odor pairs during active sensory learning and passive sensory experience (related to Figure 6).
The fate of cell-odor pairs that were initially excitatory (Ex D1), inhibitory (Inh D1) or subthreshold (Sub D1) on day 1. Data are presented as in Figure 3D.
Figure S5

Figure S5. Ensemble correlation vs. population response amplitudes and ensemble correlation in error trials (related to Figure 7).

(A) Ensemble correlation was plotted against the mean (A) or absolute difference (B) of the population Ca\(^{2+}\) responses (\(|\Delta F/F|\) values averaged across cells and during 5 s after the odor onset). Each point represents data from a given mouse on a given day (S+ and S−) or a given pair of day (S+ vs. S+ and S− vs. S−). For S+ vs. S+ and S− vs. S−, only data from a pair of different days are included.

(B) Ensemble correlation was calculated using 1) ‘Hit’: S+ trials with correct response, 2) ‘Miss’: S+ trials with incorrect response and 3) ‘CR (correct rejection)’: S− trials with correct response taken from mouse-day pairs that exceed learning threshold. Data were averaged across mouse-day pairs (black circles; n = 11 and 7 for MCs and TCs, respectively). Statistical difference was assessed with 2-way ANOVA, followed by Bonferroni’s post-hoc test (* at least \(P < 1 \times 10^{-3}\)). Bars represent mean ± SEM.
Figure S6

(A) Identification of novel odors by ensemble cortical neurons before and after passive sensory experience. Classification error rate was calculated by a linear decoder.

(B) Firing rate of the cortical cell representing S+ after passive sensory experience (top) and active learning (bottom). Responses to S+ at a low concentration (1.75) and S− at a high concentration (14) are shown in red and blue respectively. Black bar represents odor application.

(C) Ensemble correlation coefficient between S+ and S+ and between S− and S− after learning, normalized by the values before learning. Upper panels correspond to passive learning, lower panels to active learning. Experimental data presented as mean ± SEM (across mice).
Figure S7.

(A) Cumulative probability distribution of peak amplitudes was compared between GCaMP3 (black) and GCaMP6s (red) (KS test: Kolmogorov-Smirnov test). The numbers of cell-odor pairs for each indicator are shown in brackets.

(B) Distribution of response profiles. Data were processed with different methods: the response of a given cell-odor pair was considered significant when the absolute peak of trial-averaged traces exceeded twice or three times the baseline standard deviation (‘2SD’ or ‘3SD’ respectively), or when the values were significantly different between the baseline and the ON/OFF period with repeated measures ANOVA (using traces from individual trials, \( P < 0.05 \)). Data are presented as in Figure 1D (mean ± SEM). Panels for GCaMP6s 2SD were replicated from Figure 1D.
Figure S8

**Figure S8. Schema representing the implications of the findings (related to Discussion).**

MCs and TCs may receive similar glomerular inputs, but distinct inhibition from cortical feedback inputs via granule cells: MCs receive inhibition from deep granule cells (dGCs), which might be strongly modulated by cortical feedback inputs, while TCs receive inhibition from superficial granule cells (sGCs), which might be weakly modulated by cortical feedback inputs. Consequently, MCs display larger improvement of pattern separation, thereby facilitating discrimination of similar odors, whereas TCs might contribute to odor detection and/or maintenance of odor similarity information.
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Table S1. Values of all parameters used for the model (related to Figure 8 and STAR Methods).