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Bidirectional plasticity of cortical pattern recognition and behavioral sensory acuity

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Learning to adapt to a complex and fluctuating environment requires the ability to adjust neural representations of sensory stimuli. Through pattern completion processes, cortical networks can reconstruct familiar patterns from degraded input patterns, whereas pattern separation processes allow discrimination of even highly overlapping inputs. Here we show that the balance between pattern separation and completion is experience dependent. Rats given extensive training with overlapping complex odorant mixtures showed improved behavioral discrimination ability and enhanced piriform cortical ensemble pattern separation. In contrast, behavioral training to disregard normally detectable differences between overlapping mixtures resulted in impaired piriform cortical ensemble pattern separation (enhanced pattern completion) and impaired discrimination. This bidirectional effect was not found in the olfactory bulb; it may be due to plasticity within olfactory cortex itself. Thus pattern recognition, and the balance between pattern separation and completion, is highly malleable on the basis of task demands and occurs in concert with changes in perceptual performance.

The ability of some cortical circuits to fill in features missing from a familiar input pattern is known as pattern completion, a process essential to ensure perceptual stability in case of behaviorally irrelevant variations in stimuli. This mechanism gives way to pattern separation when the two input patterns become more distinct or as the significance of making discrimination between them increases. The CA3 region of the hippocampus[1,2] and more recently the anterior piriform cortex[3] (aPCX) have been revealed as critical in these computations. These two areas share functional and structural similarities and are both characterized as auto-associative memory networks[4]. The most commonly studied pattern recognition tasks in the hippocampal system involve patterns of environmental spatial cues, whereas the piriform cortex deals with odor-evoked olfactory bulb spatiotemporal patterns and the formation of odor objects[5]. In both structures, the transition between encoding patterns as similar and as different follows a nonlinear transition as stimuli morph from one pattern to another.

The goal of the present work was to test the hypothesis, derived from computational models[4,6], that shifts between pattern completion and separation would reflect not only the nature of pattern overlap but also experience and ongoing task demands. That is, tasks requiring high-acuity discrimination should shift cortical pattern recognition toward pattern separation, whereas tasks requiring stimulus grouping or reduced acuity should bias pattern recognition toward pattern completion. The olfactory cortex is an ideal model for these studies given its relatively simple architecture and proximity to the sensory epithelium, the relative ease of quantitatively manipulating sensory inputs, and the robust odor learning of rodents.

Previous work has demonstrated that odor learning and behavioral state can modulate odor-evoked responses as early as the olfactory bulb[7–9]. Here, in an effort to avoid nonspecific influences such as differential behavioral state, we recorded cortical activity in urethane-anesthetized rats 24 h after the final training session. The results suggest that piriform cortical pattern recognition is strongly shaped by experience and task demands, enhancing or degrading sensory discrimination as needed and in concert with behavioral perceptual performance.

RESULTS

Pattern completion and separation in naive rats

Data from a total of 255 cortical neurons and 104 mitral or tufted cells are included across all of the experiments reported here. Numbers of rats and units per manipulation are provided in **Supplementary Table 1**.

In odor-naive rats, we isolated single units in the olfactory bulb mitral/tufted cell layer and aPCX layer II/III and examined responses to a series of odorant mixtures. All data presented here used the same initial mixture of ten monomolecular odorants from which components were removed (for example, 10c–1, 10 components with 1 removed; 10c–2, 10 components with 2 removed) or replaced (10cR1) (**Fig. 1a**). Typical examples of aPCX and olfactory bulb single units responding to the odorant mixtures are illustrated **Supplementary Figure 1c**. These units responded to several different mixtures; however, despite the linear morphing from one mixture to the next, the response to one stimulus did not predict responsiveness to other similar, overlapping mixtures (for example, 10c and 10c–1).

We then examined how ensembles of mitral/tufted cells or aPCX neurons[3,10] differentially responded to these overlapping mixtures by studying cross-correlations between the evoked ensemble spike activity. Ensembles of neurons in the olfactory bulb decorrelated the standard 10c mix from all of the morphed versions (pattern separation) (**Fig. 1b**).

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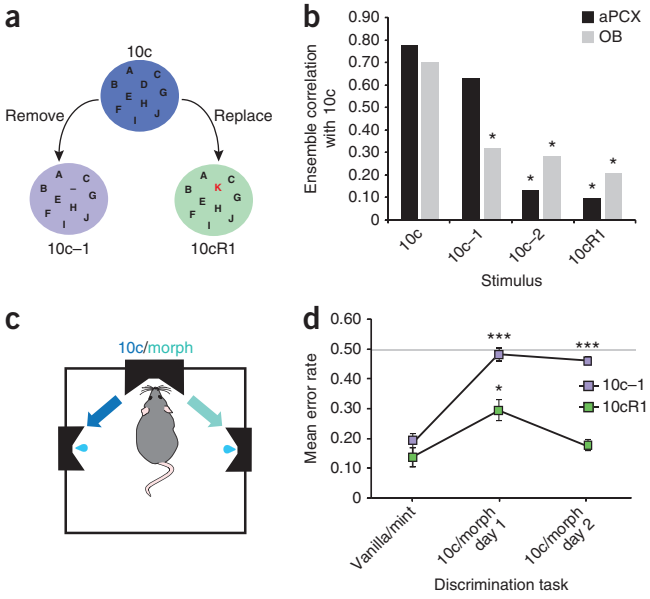

Figure 1 Sensory acuity in the naive rat. (**a**) Olfactory stimulus morphing: the original stimulus, a complex mixture of ten odorant components (10c, each component symbolized by a letter), was either degraded by the progressive removal of its components (10c–1, 10c–2, …) or transformed by the replacement of one component by another one (10cR1). (**b**) Cross-correlation analyses of olfactory bulb (OB) and of aPCX single-unit ensemble responses to the standard 10c mix versus its various morphs. OB mitral/tufted cell ensembles ($n = 28$ units) showed stable decorrelation across all morphs. aPCX pyramidal cell ensembles ($n = 20$ units) showed no decorrelation if a single component only was removed; decorrelation appeared only when more components were missing. In contrast, the addition of one single unusual component to the complex mix (10cR1) gave a clear separation of the two patterns. $^*P < 0.05$ or better, decorrelation compared to 10c. (**c**) A two-alternative forced-choice task was used to evaluate the rat's ability to discriminate the 10c full mixture from its morphed versions. (**d**) Behavioral performances matched aPCX discrimination capacities. Rats could not detect the removal of a single component (purple), whereas they could easily discriminate mixtures in which a novel component had been introduced (green). $^*P < 0.05$ and $^{***}P < 0.001$ for each group versus the respective reference performance (vanilla/mint). Error bars, s.e.m.

In contrast, aPCX ensembles showed no significant decorrelation if only a single component was missing (90% overlap; difference in ensemble correlation between 10c and 10c–1, $Z = 0.866$, $P = 0.1933$) and responded as if the full mixture were present (pattern completion); decorrelation increased as more components were removed (for 80% overlap, difference in ensemble correlation between 10c and 10c–2, $Z = 2.617$, $P = 0.0044$) (pattern separation). Furthermore, aPCX ensembles strongly decorrelated the 10c mix from the same mixture with one component replaced (10cR1; $Z = 2.734$, $P = 0.0031$) (pattern separation).

We trained different naive rats in a two-alternative forced-choice task to evaluate their ability to discriminate the 10c mixture from its morphed versions (**Fig. 1c**). Discrete manipulations of the standard 10c mix markedly changed discrimination performance (**Fig. 1d**). After 2 d of training, the rats could discriminate the standard mix from the same mixture with one component replaced (10c/10cR1), whereas after the same period of time all rats failed to detect the removal of one component from the standard (10c/10c–1). The behavioral sensory acuity in naive rats thus matched aPCX ensemble pattern discrimination.

Experience increases pattern separation and acuity

Rats were trained in either an 'easy' 10c/10cR1 discrimination until reaching performance criterion (error rate <0.25, 2 d of training; **Fig. 1d**) or in the more difficult 10c/10c–1 task until reaching criterion (8 d of training; **Fig. 2a**). Other rats were also trained in the easy 10c/10cR1 discrimination for 8 d (**Fig. 2a**) to match the duration of training in the difficult task. We recorded single unit-activity in olfactory bulb and aPCX 24 h after the last training session. No change was observed in single-unit spontaneous activity after training, although there was a significant decrease in the probability of odor-evoked activity in rats trained in the difficult task (see **Supplementary Results** and **Discussion**).

Learning the difficult behavioral discrimination was associated with enhanced stimulus decorrelation (pattern separation) in aPCX ensembles (**Fig. 2b**). In rats trained 2 d in the easy discrimination task, aPCX ensembles showed a mixture decorrelation comparable to that in naive rats. In contrast, in rats successfully trained to make the difficult discrimination (10c/10c–1), cortical decorrelation for this comparison was strongly significant ($Z = -3.128$, $P = 0.0009$). Thus, behavioral conditioning was associated with an enhancement in pattern separation in aPCX for odors having formerly pronounced qualitative and cortical encoding similarities. Notably, no improvement in mixture decorrelation was observed in the rats trained in the longer version of the easy training (10c still correlated to 10c–1; $Z = 0.603$, $P = 0.2733$). Finally, in the olfactory bulb, mitral/tufted ensembles showed stable pattern separation after the difficult discrimination task compared to that in naive rats, suggesting a cortical origin for the observed changes in aPCX activity.

We also examined the effect of experience on breadth of tuning of single units (**Fig. 2c**). Breadth of tuning was measured with entropy (Online Methods), which ranges from a value of 0, where a single unit responds selectively to only one stimulus in a test set (here 10c, 10c–1, 10c–2, 10cR1), to 1, where a single unit responds equally well to all stimuli in the test set. In aPCX, the entropy value for units in rats trained in the easy discrimination task did not differ from that of naive rats (short training: $t_{41} = -1.384$, $P = 0.1737$; long training: $t_{46} = 0.026$, $P = 0.9797$), whereas in rats trained in the difficult task, entropy was significantly lower ($t_{37} = -2.714$, $P = 0.0100$) (**Fig. 2c**). This result indicates that aPCX neurons were more narrowly tuned in rats mastering a difficult (but not an easy) olfactory discrimination. In olfactory bulb, the same difficult learning did not change the selectivity of mitral/tufted cells ($t_{42} = 0.471$, $P = 0.6402$).

In addition to single-unit activity, we also assessed whether training induced changes within olfactory bulb and aPCX networks through examination of local field potentials (LFPs). In the naive anesthetized rat, mixtures evoked pronounced oscillatory activities in the beta (15–35 Hz) and gamma (40–80 Hz) frequency range in both olfactory bulb and aPCX (**Supplementary Fig. 1d**). In rats trained in the easy discrimination task for either 2 or 8 d, we observed no change of power in the beta or gamma bands compared to that in naive rats, for all odorant morphs tested (Mann-Whitney U test, $P > 0.05$). In contrast, in rats trained in the difficult discrimination task, aPCX beta oscillations were strongly enhanced (pooled across all odorants, $U_{548} = 27{,}341$, $P < 0.0001$). More precisely, aPCX beta oscillations were selectively enhanced in response to the learned odors (10c: $U_{137} = 1{,}317$, $P < 0.0001$; 10c–1: $U_{135} = 1{,}494$, $P = 0.0003$) but not in response to the unfamiliar morphs (10c–2: $U_{136} = 1{,}999$, $P = 0.1152$; 10cR1: $U_{134} = 1{,}866$, $P = 0.0585$). No change in beta power occurred in olfactory bulb (**Fig. 2d**, $U_{649} = 48{,}105$, $P = 0.3229$). Odor-evoked

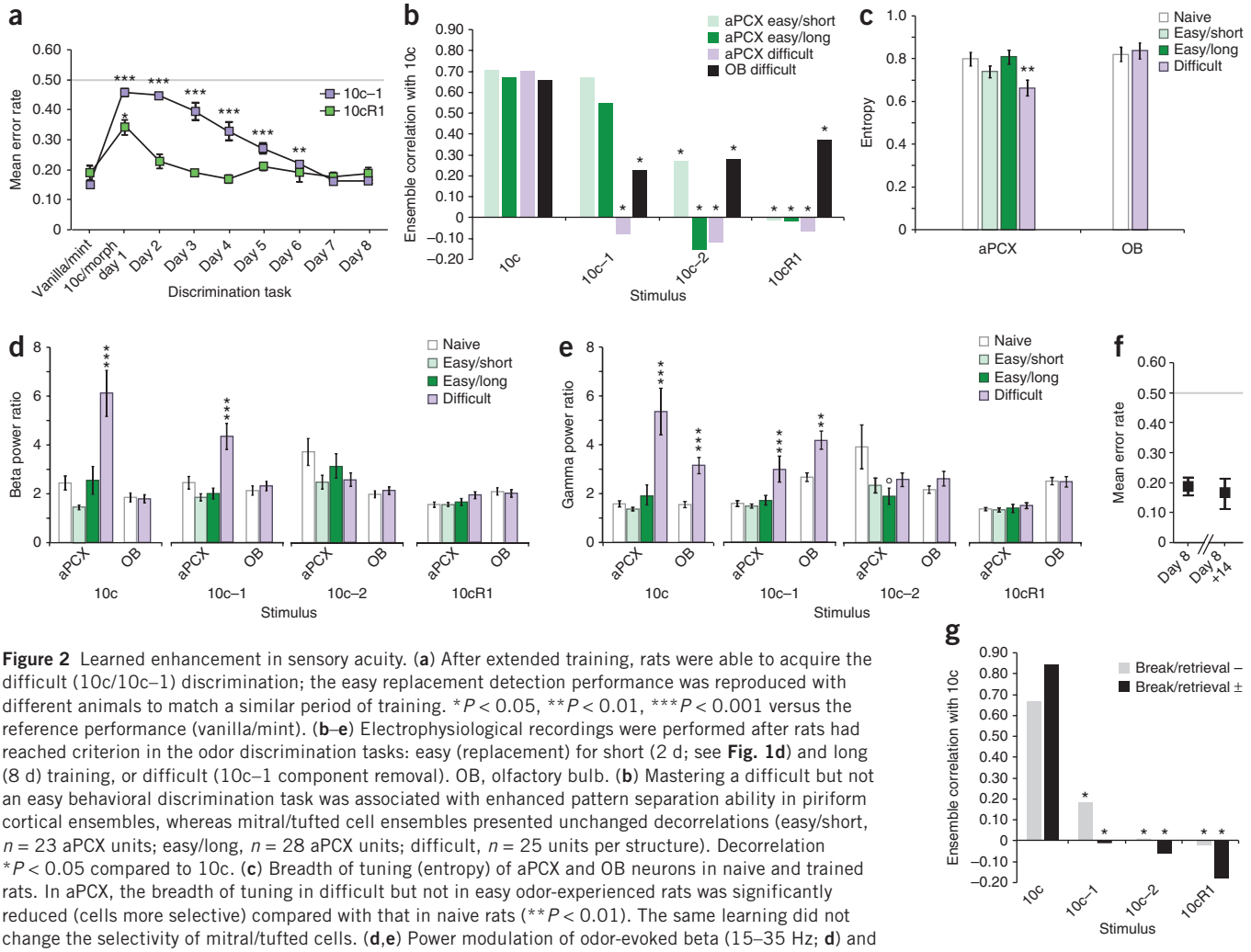

Figure 2 Learned enhancement in sensory acuity. (**a**) After extended training, rats were able to acquire the difficult (10c/10c–1) discrimination; the easy replacement detection performance was reproduced with different animals to match a similar period of training. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the reference performance (vanilla/mint). (**b**–**e**) Electrophysiological recordings were performed after rats had reached criterion in the odor discrimination tasks: easy (replacement) for short (2 d; see **Fig. 1d**) and long (8 d) training, or difficult (10c–1 component removal). OB, olfactory bulb. (**b**) Mastering a difficult but not an easy behavioral discrimination task was associated with enhanced pattern separation ability in piriform cortical ensembles, whereas mitral/tufted cell ensembles presented unchanged decorrelations (easy/short, $n = 23$ aPCX units; easy/long, $n = 28$ aPCX units; difficult, $n = 25$ units per structure). Decorrelation $^{*}P < 0.05$ compared to 10c. (**c**) Breadth of tuning (entropy) of aPCX and OB neurons in naive and trained rats. In aPCX, the breadth of tuning in difficult but not in easy odor-experienced rats was significantly reduced (cells more selective) compared with that in naive rats ($^{**}P < 0.01$). The same learning did not change the selectivity of mitral/tufted cells. (**d**,**e**) Power modulation of odor-evoked beta (15–35 Hz; **d**) and gamma (40–80 Hz; **e**) oscillatory activities after discrimination learning. Mastering the difficult discrimination task was associated with enhanced gamma and beta odor-evoked activity compared to the simple task and naive status. $n = 212$–296 odor-evoked responses per structure and per experimental group. $^{**}P < 0.01$ and $^{***}P < 0.001$, higher power than in naive rats; $^{\circ}P < 0.05$, lower power than in naive rats. (**f**,**g**) Long-lasting memory of the difficult discrimination training. (**f**) Maintenance of the behavioral discrimination after a 2-week break in the training. (**g**) Maintenance of aPCX neurons pattern separation ability after a 2-week break in the training, either without behavioral retrieval test (gray, $n = 25$ cells) or with and without retrieval test (black, $n = 35$ cells). $^{*}P < 0.05$ compared to 10c. Data are shown as mean ± s.e.m.

gamma band activity (**Fig. 2e**) showed comparable enhancements of power in aPCX (pooled odorants, $U_{548} = 24{,}675$, $P < 0.0001$) and olfactory bulb ($U_{649} = 39{,}682$, $P < 0.0001$). For both structures, this enhancement was selective in response to the learned odors (aPCX: 10c: $U_{137} = 703$, $P < 0.0001$; 10c–1: $U_{135} = 1{,}090$, $P < 0.0001$; OB: 10c: $U_{163} = 1{,}835$, $P < 0.0001$; 10c–1: $U_{159} = 2{,}325$, $P = 0.0097$).

Odor discrimination training can evoke both transient and long-term changes in olfactory cortical activity[11,12]. To determine whether training induced a long-term change in cortical pattern separation, other rats ($n = 5$) were trained in the difficult 10c/10c–1 discrimination task until reaching criterion (similarly to **Fig. 2a**). We then imposed a break of 14 d without training or odor exposure. Long-term memory was assessed afterward for some of the rats ($n = 2$). As shown in **Figure 2f**, their performance after the break showed no decrement compared to the last day of the pre-break training. In parallel, the other rats ($n = 3$) were directly recorded after the break (without new training) to avoid any reconsolidation effect that the post-break retrieval test might have induced. The aPCX neural ensemble responses obtained for these rats verify a strong cortical decorrelation between 10c and its morphed versions, similar to that obtained when the recordings occurred 24 h after training (break/retrieval–, **Fig. 2g**). The same pattern of results was obtained if the analysis pooled the cortical recordings of both behaviorally tested and untested rats (break/retrieval ±). However, although behavioral and ensemble changes lasted for at least 2 weeks, learned changes in odor-evoked oscillations in aPCX did not (see **Supplementary Fig. 2**), consistent with a more transient role for these processes during early stages of conditioning and memory[13].

Experience increases pattern completion and generalization

Can rats learn to ignore differences between odors and in so doing impair olfactory cortical acuity by enhancing pattern completion? To address this question, we developed a paradigm wherein high olfactory acuity impairs performance in the behavioral task. In this modified two-alternative forced-choice task, two odors A and A′ were associated to the same rewarded side in opposition to a third odor B, rewarded on the other side (**Fig. 3a**). We tested two levels of olfactory similarity between A and A′. In a first condition ('close', **Fig. 3b**),

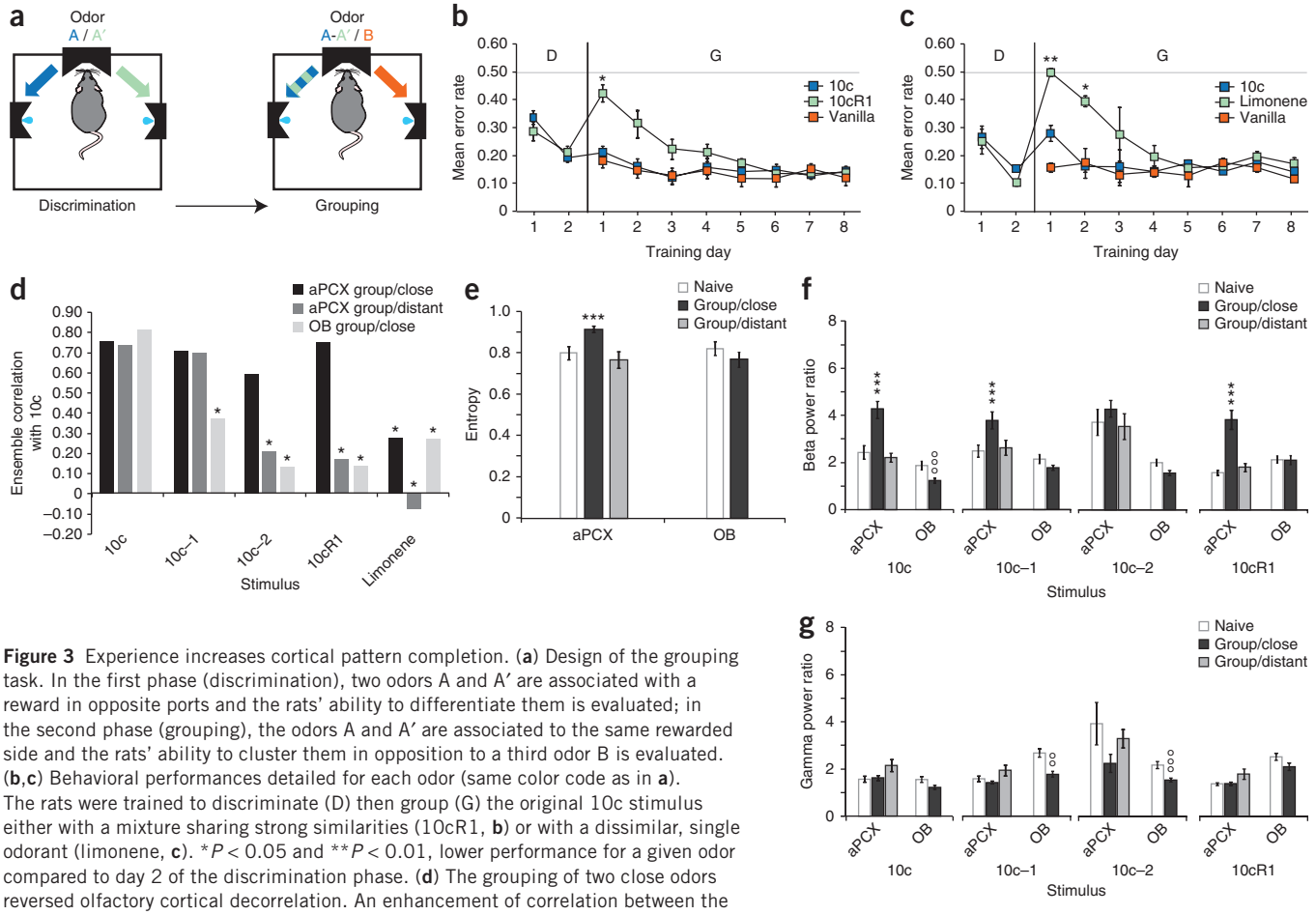

Figure 3 Experience increases cortical pattern completion. (**a**) Design of the grouping task. In the first phase (discrimination), two odors A and A′ are associated with a reward in opposite ports and the rats' ability to differentiate them is evaluated; in the second phase (grouping), the odors A and A′ are associated to the same rewarded side and the rats' ability to cluster them in opposition to a third odor B is evaluated. (**b**,**c**) Behavioral performances detailed for each odor (same color code as in **a**). The rats were trained to discriminate (D) then group (G) the original 10c stimulus either with a mixture sharing strong similarities (10cR1, **b**) or with a dissimilar, single odorant (limonene, **c**). $^{*}P < 0.05$ and $^{**}P < 0.01$, lower performance for a given odor compared to day 2 of the discrimination phase. (**d**) The grouping of two close odors reversed olfactory cortical decorrelation. An enhancement of correlation between the full 10c mix and all its morphed versions was obtained in rats trained to group close stimuli ($n = 31$ aPCX units). aPCX discrimination capacities of rats trained to group distant stimuli corresponded to those of naive rats ($n = 19$ aPCX units). The grouping of close odors did not change olfactory bulb (OB) pattern separation ability ($n = 24$ mitral/tufted cells). $^{*}P < 0.05$, significant decorrelation compared to 10c. (**e**) The breadth of tuning of aPCX neurons in rats trained to group close but not distant odors was significantly higher (cells less selective) than that in naive rats ($^{***}P < 0.005$). Mitral/tufted cells selectivity did not differ from naive rats after the grouping task. (**f**,**g**) Power modulation of odor-evoked beta (**f**) and gamma (**g**) oscillatory activities associated to grouping learning in aPCX and OB. $n = 156$–372 odor-evoked responses per structure and per experimental group. $^{***}P < 0.001$, higher power than in naive rats; $^{°°}P < 0.01$ and $^{°°°}P < 0.001$, lower power than in naive rats. Data are shown as mean ± s.e.m.

the rats had to group two highly similar but nonetheless discernible mixtures (10c and 10cR1). In the second condition ('distant', **Fig. 3c**), different rats had to group two very dissimilar odors (10c and limonene, a monomolecular odorant). Rats learned the two tasks in comparable amounts of time.

aPCX cell ensemble decorrelation was strongly affected in a task-specific manner (**Fig. 3d**). In the rats trained to group close mixtures, we observed an increase in correlation between the standard and its transformed versions, particularly with 10c–2 ($Z = 1.19$, $P = 0.1171$) and 10cR1 ($Z = 0.035$, $P = 0.4859$), indicating that aPCX neural ensembles were able to reduce divergence of mixture encoding (pattern completion) when high olfactory acuity would have impaired behavioral performance. This suggests that aPCX ensembles merged the representation of two, formerly discriminable overlapping mixtures (10c and 10cR1) into a single representation that signaled a left reward. Note that aPCX ensembles still decorrelated 10c from a monomolecular element not present in the original mix (limonene, $Z = 2.703$, $P = 0.0034$), indicating that the observed increase in pattern completion and decrease in decorrelation were limited to similar, overlapping input patterns. Furthermore, in the rats trained to group distant odors, aPCX ensemble decorrelation was comparable to that in the naive condition. This suggests that in this version of the task the rats solved the problem by learning that either of two distinct odors signaled a left reward, rather than merging the representations of the two. In contrast to that in the aPCX, olfactory bulb mitral/tufted cell ensemble decorrelation was not modified for rats trained to group close odor mixtures.

The mean breadth of tuning of single units (**Fig. 3e**) in rats trained to group close mixtures was significantly higher than that in the naive condition ($t_{45} = -3.351$, $P = 0.0016$), whereas there was no change in selectivity when the grouping involved distant odors ($t_{40} = -0.658$, $P = 0.5145$), showing that aPCX neurons were more broadly tuned when the rats were trained to ignore the differences between similar (but not dissimilar) olfactory stimuli. We observed no change in the selectivity of mitral/tufted cells in the close version of the grouping task ($t_{48} = -1.332$, $P = 0.1892$). Further measures of changes in single-unit activity are presented in the **Supplementary Results** and **Discussion**. Finally, LFP analysis revealed a significant elevation of aPCX beta power in the rats trained to group close odors (pooled odorants, $U_{603} = 29{,}102$, $P < 0.0001$), in response to the learned mixtures (10c: $U_{154} = 1{,}788$, $P < 0.0001$; 10cR1: $U_{148} = 1{,}342$, $P < 0.0001$) but also in response to some unfamiliar but overlapping versions

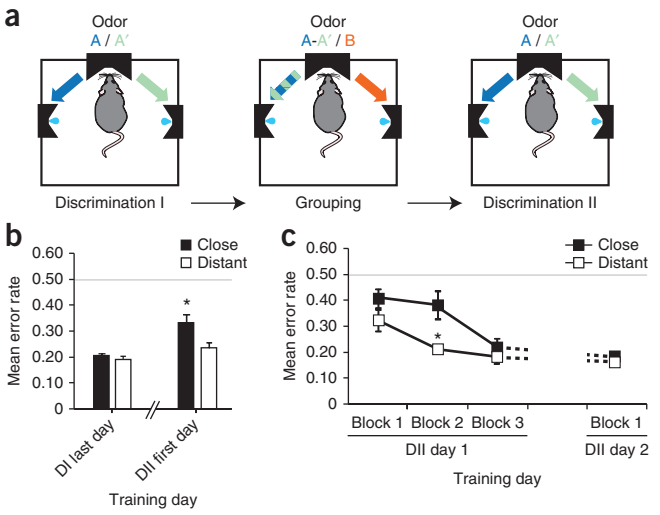

Figure 4 Learned enhancement in sensory generalization. (**a**) Behavioral training. The rats' discrimination capacity was evaluated again after achievement of the grouping task. A = 10c; A′ = 10cR1 ('close') or limonene ('distant'); B = vanilla. (**b**) Averaged performances. The rats were poorer at discriminating close odors after training in the grouping task than they were before (*$P < 0.05$); this impairment was not observed when the grouping task involved distant odors. (**c**) Detailed performances over time. The error rate of the discrimination session after the grouping task is divided into three consecutive blocks of trials. *$P < 0.05$, lower performance for the rats trained to group close odors than for those trained to group distant. Data are shown as mean ± s.e.m.

(10c–1, $U_{148} = 1{,}913$, $P = 0.0014$) (**Fig. 3f**). We observed no change in beta power in aPCX in the rats trained to group distant odors (Mann-Whitney U test, $P > 0.05$). No significant change of odor-evoked gamma power was observed after the grouping task regardless of the proximity of the odors used (Mann-Whitney U test, $P > 0.05$) (**Fig. 3g**). Compared to naive rats, we observed a global decrease of power in response to the mixtures in the olfactory bulb both in the beta (all odorants considered, $U_{626} = 38{,}952$, $P = 0.0016$) and gamma frequency bands (all odorants considered, $U_{626} = 37{,}783$, $P = 0.0002$). A notable decrease was particularly observed in the beta frequency band for 10c ($U_{156} = 1{,}800$, $P < 0.0001$) and in the gamma band for 10c–1 ($U_{156} = 2{,}228$, $P = 0.0128$) and 10c–2 ($U_{155} = 1{,}788$, $P = 0.0001$).

The data presented above demonstrate a shift toward enhanced cortical generalization due to the olfactory grouping learning. To identify whether this change was accompanied by a behavioral impairment in discriminating the two close mixtures (10c and 10cR1), other rats were trained in the grouping task for 8 d as previously described (A-A′ versus B) but were then returned to the original two-alternative forced-choice task (A versus A′) to re-measure their discriminative acuity (**Fig. 4a**). Results (**Fig. 4b**) indicated a significant impairment in discriminating 10c from 10cR1 after the grouping task compared to before (paired t test, $t_3 = -3.604$, $P = 0.0366$). In contrast, the rats

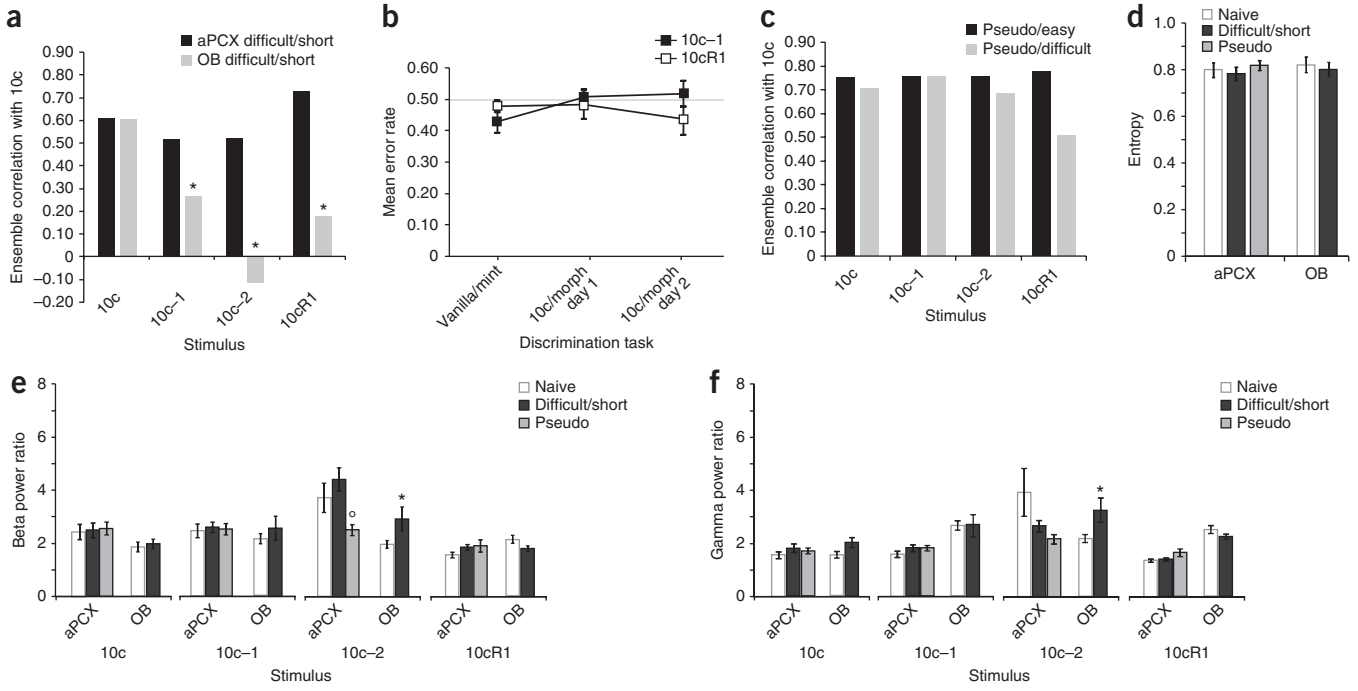

Figure 5 Transient decrease in sensory acuity associated with poor behavioral performance. (**a**) Electrophysiological recordings were performed at the early stage of the difficult task in rats not yet able to perform the 10c/10c–1 discrimination after a short (2 d) training (see **Fig. 1d**). Cross-correlation analysis for aPCX ensembles showed high correlations between the 10c full mixture and all its morphed versions, whereas olfactory bulb (OB) decorrelation capacities were unaltered by the same experience ($n = 21$ and 27 aPCX and OB units, respectively). *$P < 0.05$, decorrelation compared to 10c. (**b**) The pseudo-conditioned rats never reached the criterion of the reference performance (error rate <0.25 for vanilla/mint) and were merely exposed, without behavioral consequence, to the same pairs of complex mixtures as the conditioned rats. (**c**) Cross-correlation analysis for aPCX ensembles in the pseudo-conditioned rats (pseudo) submitted to easy-like ($n = 27$ units) or difficult-like ($n = 26$ units) discrimination tasks showed significant correlations between the 10c mix and all its morphed versions. (**d**) Breadth of tuning. Olfactory bulb and aPCX neurons recorded in rats either at the early stage of the difficult discrimination training or pseudo-conditioned showed comparable odor selectivity to those in naive rats. (**e**,**f**) Power modulation of odor-evoked beta (**e**) and gamma (**f**) oscillatory activities associated to poor behavioral performances. $n = 212$–285 odor-evoked responses per structure and per experimental group. *$P < 0.05$, higher power than in naive rats; °$P < 0.05$, lower power than in naive rats. Data are shown as mean ± s.e.m.

that were trained in the grouping task involving distant odors showed no significant decline in discriminating 10c from limonene ($t_2 = -1.688$, $P = 0.2335$). Repeated-measures ANOVA indicated a significant effect of trial block ($F_{1,3} = 25.951$, $P < 0.0001$) but no significant effect of group ($F_{1,5} = 4.479$, $P = 0.0879$), showing that the two groups improved their performance over the course of the session (**Fig. 4c**). However, there was a significant block × group interaction ($F_{1,3} = 4.121$, $P = 0.0256$): whereas the rats in the distant odor group were already at criterion by the second block of trials, the rats in the close odor group were significantly poorer for this same block (unpaired *t* test, $t_5 = 2.686$, $P = 0.0435$). Together, these results suggest an impairment of olfactory acuity induced by the close version of the grouping task. After this training, and as long as it remained behaviorally relevant, the animal treated the two stimuli (10c and 10cR1) as the same odor object both at the piriform cortical and the behavioral level.

Cortical pattern recognition during poor performance

How are olfactory bulb and aPCX ensemble activity affected during periods of poor discrimination performance? We considered two distinct situations. The first focused on the early stages of the difficult discrimination task, when the rats could not yet distinguish 10c from10c–1: we isolated olfactory bulb and aPCX units from rats trained in this task when performance was still at chance levels (2 d of training; **Figs. 1d** and **5a**). The second situation involved rats that failed to master the simple discrimination task of the pre-training (vanilla/mint) and were thus exposed to the complex mixtures but did not use them to guide their choice behavior (pseudo-conditioning). Although the animals performed at chance levels (**Fig. 5b**), they were stimulated with the same pairs of odorant mixtures (either easy-like (10c/10cR1) or difficult-like (10c/10c–1) discriminations) and for the same period of time (2 d) as the conditioned rats. We did ensure that these rats were not anosmic, as they were able to detect the introduction of novel odors in the cage (data not shown) and their aPCX neurons showed normal activity in response to various odorants (**Fig. 5c**).

After 2 d of training in the difficult discrimination task, at a time when rats performed at chance levels, aPCX ensembles showed a strong increase in pattern completion, failing to decorrelate any of the overlapping mixtures, especially 10c–2 ($Z = 0.395$, $P = 0.3464$) and 10cR1 ($Z = -0.669$, $P = 0.2518$) (**Fig. 5a**), whereas for the same situation olfactory bulb mitral/tufted cell ensembles decorrelated the odor mixtures similarly to those in naive rats.

We obtained analogous enhancements of piriform cortical correlations in pseudo-conditioned rats, regardless of the pair of mixtures to which they had been exposed (easy- or difficult-like) (**Fig. 5c**). These results suggest a potential, experience-dependent 'resetting' of cortical acuity during failed discrimination performance, perhaps through a change in cell excitability[14] or cortical synaptic inhibition[15] that may be required for subsequent refinement in pattern recognition. Of note, this failure of ensemble decorrelation during random discrimination performance was not associated with a change in single-unit tuning selectivity (**Fig. 5d**). Finally, the analysis of LFPs revealed no consistent, significant difference in power of the odor-evoked beta (**Fig. 5e**) or gamma frequency bands (**Fig. 5f**) for the rats showing poor discrimination performance compared to beta and gamma power in naive rats.

DISCUSSION

Experience-dependent pattern recognition by networks such as the hippocampus and piriform cortex promotes both perceptual stability through pattern completion and perceptual discrimination through pattern separation. The memory of previously experienced patterns, through changes in synaptic weights within the network[16,17], is a critical component of these divergent pattern recognition processes. The present results suggest that the balance between pattern separation and pattern completion is also experience dependent. A task requiring discrimination of highly overlapping patterns induces changes in cortical activity that shift the balance toward pattern separation (enhanced decorrelation) and enhance perceptual acuity. A task requiring disregard of differences between overlapping patterns for optimal performance induces changes in cortical activity that shift the balance toward pattern completion (enhanced correlation) and impair perceptual acuity. Training in a simple task, in contrast, produces none of these changes in cortical processing or perception (summarized in **Supplementary Fig. 3**). Importantly, although as shown here and elsewhere[18–22], odor learning induces changes in olfactory bulb odor responses, the learned changes in piriform cortical ensembles were not associated with similar changes in the olfactory bulb ensembles, suggesting a piriform localization.

The task-dependent changes in cortical ensemble correlation and decorrelation were associated with changes in the breadth of single-unit odor receptive fields. Such cortical receptive field changes have been observed in several systems[23,24], including the aPCX[25]. The breadth of tuning of neurons in many sensory systems is modulated by local inhibition. There are several classes of inhibitory interneurons in the aPCX[26] mediating both feedforward and feedback inhibition[27]. Learning induced plasticity of these neurons[15] could contribute to pyramidal cell receptive field changes. Finally, there are several classes of pyramidal cells in aPCX layers II and III with different connectivity to afferent and association fiber pathways[28]. These cells may be differentially modified by learning.

Notably, during periods of impaired performance, cortical ensembles also shift toward pattern completion. Learned impairments in perception have also been described in humans[29]. These highly correlated ensemble responses may reflect the increased excitability observed in aPCX neurons during early stages of odor discrimination training[14], though we observed no increase in spontaneous or odor evoked activity. Nonetheless, enhanced excitability may facilitate opportunities for associative synaptic plasticity that could subsequently lead to the refinement of ensemble responses and improvement in perceptual acuity in later phases of training. Top-down or modulatory inputs to aPCX providing feedback regarding response outcomes may also be necessary for induction and expression of the learned changes[16,30].

In addition to changes in single-unit and ensemble plasticity, odor-evoked olfactory system LFPs were also modified by training. Although olfactory system odor-evoked activity is strongly modulated by anesthesia[31] and state[32], the demonstration here in anesthetized rats that olfactory system beta and gamma oscillations reflect the rat's past olfactory experience and are selectively increased in difficult tasks match well with recordings in awake rats[33,34]. Oscillations have been shown to support sensory perception and neural plasticity and to allow transient cooperation between cerebral areas[35]. Gamma-frequency oscillations tend to reflect local circuit activity, whereas beta oscillations reflect more widespread network activity[36,37]. In the olfactory system, oscillations have been suggested to facilitate precise temporal structure of spike trains[38], which may be expected to modulate spike timing–dependent plasticity in downstream targets[21]. Inhibitory interneurons are important in the temporal structure of oscillations[39] and thus may be implicated in learned changes. Of note, whereas odor decorrelation by cortical ensembles was long-lasting (2 weeks), learned changes in oscillations were more transient and were not detected after a 2-week delay. This suggests substantially

different roles for these two measures of circuit function (oscillations and ensemble decorrelation) in storing sensory memory.

Finally, these results suggest that one consequence of learning to ignore differences between patterns is an impairment in subsequent sensory discrimination. Olfactory impairments are associated with a variety of neuropathological conditions, ranging from schizophrenia[40] to Alzheimer's disease[41]. Similar pathology- or aging-related impairments in hippocampal pattern separation have also been reported[42–44]. The olfactory perceptual impairments have been associated with pathology within the olfactory system[45,46]. However, if the pathology modifies cognitive aspects that lead to ignoring differences between stimuli, the present results suggest that an extra factor exacerbating perceptual decline could be an experience-dependent remodeling of cortical ensembles and a learned impairment in discrimination. Thus, the balance between pattern completion and separation, which can be shifted depending on task demands, could instead serve in disease states to impair perception and memory.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

D.A.W. and J.C. designed the research. J.C. collected data. J.C. and D.A.W. analyzed and interpreted data. J.C. and D.A.W. wrote the paper.

COMPETING FINANCIAL INTERESTS

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1. Lee, I., Yoganarasimha, D., Rao, G. & Knierim, J.J. Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. *Nature* **430**, 456–459 (2004).
2. Leutgeb, S., Leutgeb, J.K., Treves, A., Moser, M.B. & Moser, E.I. Distinct ensemble codes in hippocampal areas CA3 and CA1. *Science* **305**, 1295–1298 (2004).
3. Barnes, D.C., Hofacer, R.D., Zaman, A.R., Rennaker, R.L. & Wilson, D.A. Olfactory perceptual stability and discrimination. *Nat. Neurosci.* **11**, 1378–1380 (2008).
4. Haberly, L.B. Parallel-distributed processing in olfactory cortex: new insights from morphological and physiological analysis of neuronal circuitry. *Chem. Senses* **26**, 551–576 (2001).
5. Wilson, D.A. & Stevenson, R.J. The fundamental role of memory in olfactory perception. *Trends Neurosci.* **26**, 243–247 (2003).
6. McClelland, J.L. & Goddard, N.H. Considerations arising from a complementary learning systems perspective on hippocampus and neocortex. *Hippocampus* **6**, 654–665 (1996).
7. Wesson, D.W., Donahou, T.N., Johnson, M.O. & Wachowiak, M. Sniffing behavior of mice during performance in odor-guided tasks. *Chem. Senses* **33**, 581–596 (2008).
8. Fletcher, M.L. & Chen, W.R. Neural correlates of olfactory learning: Critical role of centrifugal neuromodulation. *Learn. Mem.* **17**, 561–570 (2010).
9. Restrepo, D., Doucette, W., Whitesell, J.D., McTavish, T.S. & Salcedo, E. From the top down: flexible reading of a fragmented odor map. *Trends Neurosci.* **32**, 525–531 (2009).
10. Kadohisa, M. & Wilson, D.A. Separate encoding of identity and similarity of complex familiar odors in piriform cortex. *Proc. Natl. Acad. Sci. USA* **103**, 15206–15211 (2006).
11. Saar, D., Grossman, Y. & Barkai, E. Reduced synaptic facilitation between pyramidal neurons in the piriform cortex after odor learning. *J. Neurosci.* **19**, 8616–8622 (1999).
12. Saar, D. & Barkai, E. Long-term modifications in intrinsic neuronal properties and rule learning in rats. *Eur. J. Neurosci.* **17**, 2727–2734 (2003).
13. Martin, C., Beshel, J. & Kay, L.M. An olfacto-hippocampal network is dynamically involved in odor-discrimination learning. *J. Neurophysiol.* **98**, 2196–2205 (2007).
14. Cohen-Matsliah, S.I., Rosenblum, K. & Barkai, E. Olfactory-learning abilities are correlated with the rate by which intrinsic neuronal excitability is modulated in the piriform cortex. *Eur. J. Neurosci.* **30**, 1339–1348 (2009).
15. Brosh, I. & Barkai, E. Learning-induced enhancement of feedback inhibitory synaptic transmission. *Learn. Mem.* **16**, 413–416 (2009).
16. Hasselmo, M.E., Anderson, B.P. & Bower, J.M. Cholinergic modulation of cortical associative memory function. *J. Neurophysiol.* **67**, 1230–1246 (1992).
17. Nakazawa, K. *et al.* Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science* **297**, 211–218 (2002).
18. Freeman, W.J. & Schneider, W. Changes in spatial patterns of rabbit olfactory EEG with conditioning to odors. *Psychophysiology* **19**, 44–56 (1982).
19. Fletcher, M.L. & Wilson, D.A. Olfactory bulb mitral-tufted cell plasticity: odorant-specific tuning reflects previous odorant exposure. *J. Neurosci.* **23**, 6946–6955 (2003).
20. Doucette, W. & Restrepo, D. Profound context-dependent plasticity of mitral cell responses in olfactory bulb. *PLoS Biol.* **6**, e258 (2008).
21. Doucette, W. *et al.* Associative cortex features in the first olfactory brain relay station. *Neuron* **69**, 1176–1187 (2011).
22. Jones, S.V., Choi, D.C., Davis, M. & Ressler, K.J. Learning-dependent structural plasticity in the adult olfactory pathway. *J. Neurosci.* **28**, 13106–13111 (2008).
23. Bakin, J.S., Lepan, B. & Weinberger, N.M. Sensitization induced receptive field plasticity in the auditory cortex is independent of CS-modality. *Brain Res.* **577**, 226–235 (1992).
24. Kilgard, M.P. & Merzenich, M.M. Cortical map reorganization enabled by nucleus basalis activity. *Science* **279**, 1714–1718 (1998).
25. Chen, C.F., Barnes, D.C. & Wilson, D.A. Generalized versus stimulus-specific learned fear differentially modifies stimulus encoding in primary sensory cortex of awake rats. *J. Neurophysiol.* doi:10.1152/jn.00721.2011 (14 September 2011).
26. Suzuki, N. & Bekkers, J.M. Inhibitory neurons in the anterior piriform cortex of the mouse: classification using molecular markers. *J. Comp. Neurol.* **518**, 1670–1687 (2010).
27. Stokes, C.C. & Isaacson, J.S. From dendrite to soma: dynamic routing of inhibition by complementary interneuron microcircuits in olfactory cortex. *Neuron* **67**, 452–465 (2010).
28. Suzuki, N. & Bekkers, J.M. Two layers of synaptic processing by principal neurons in piriform cortex. *J. Neurosci.* **31**, 2156–2166 (2011).
29. Resnik, J., Sobel, N. & Paz, R. Auditory aversive learning increases discrimination thresholds. *Nat. Neurosci.* **14**, 791–796 (2011).
30. Li, F., Wang, L.P., Shen, X. & Tsien, J.Z. Balanced dopamine is critical for pattern completion during associative memory recall. *PLoS ONE* **5**, e15401 (2010).
31. Rinberg, D., Koulakov, A. & Gelperin, A. Sparse odor coding in awake behaving mice. *J. Neurosci.* **26**, 8857–8865 (2006).
32. Barnes, D.C., Chapuis, J., Chaudhury, D. & Wilson, D.A. Odor fear conditioning modifies piriform cortex local field potentials both during conditioning and during post-conditioning sleep. *PLoS ONE* **6**, e18130 (2011).
33. Beshel, J., Kopell, N. & Kay, L.M. Olfactory bulb gamma oscillations are enhanced with task demands. *J. Neurosci.* **27**, 8358–8365 (2007).
34. Kay, L.M. & Beshel, J. A beta oscillation network in the rat olfactory system during a 2-alternative choice odor discrimination task. *J. Neurophysiol.* **104**, 829–839 (2010).
35. Fell, J. & Axmacher, N. The role of phase synchronization in memory processes. *Nat. Rev. Neurosci.* **12**, 105–118 (2011).
36. Kay, L.M. *et al.* Olfactory oscillations: the what, how and what for. *Trends Neurosci.* **32**, 207–214 (2009).
37. Chapuis, J. *et al.* The way an odor is experienced during aversive conditioning determines the extent of the network recruited during retrieval: a multisite electrophysiological study in rats. *J. Neurosci.* **29**, 10287–10298 (2009).
38. Cenier, T. *et al.* Respiration-gated formation of gamma and beta neural assemblies in the mammalian olfactory bulb. *Eur. J. Neurosci.* **29**, 921–930 (2009).
39. Nusser, Z., Kay, L.M., Laurent, G., Homanics, G.E. & Mody, I. Disruption of GABA(A) receptors on GABAergic interneurons leads to increased oscillatory power in the olfactory bulb network. *J. Neurophysiol.* **86**, 2823–2833 (2001).
40. Seckinger, R.A. *et al.* Olfactory identification and WAIS-R performance in deficit and nondeficit schizophrenia. *Schizophr. Res.* **69**, 55–65 (2004).
41. Murphy, C. Loss of olfactory function in dementing disease. *Physiol. Behav.* **66**, 177–182 (1999).
42. Tanila, H., Shapiro, M., Gallagher, M. & Eichenbaum, H. Brain aging: changes in the nature of information coding by the hippocampus. *J. Neurosci.* **17**, 5155–5166 (1997).
43. Tanila, H., Sipila, P., Shapiro, M. & Eichenbaum, H. Brain aging: impaired coding of novel environmental cues. *J. Neurosci.* **17**, 5167–5174 (1997).
44. Barnes, C.A., Suster, M.S., Shen, J. & McNaughton, B.L. Multistability of cognitive maps in the hippocampus of old rats. *Nature* **388**, 272–275 (1997).
45. Wesson, D.W., Levy, E., Nixon, R.A. & Wilson, D.A. Olfactory dysfunction correlates with amyloid-beta burden in an Alzheimer's disease mouse model. *J. Neurosci.* **30**, 505–514 (2010).
46. Li, W., Howard, J.D. & Gottfried, J.A. Disruption of odour quality coding in piriform cortex mediates olfactory deficits in Alzheimer's disease. *Brain* **133**, 2714–2726 (2010).

ONLINE METHODS

Subjects. All experiments were conducted in accordance with the guidelines of the US National Institutes of Health and were approved by the Institutional Animal Care and Use Committees at both the Nathan Kline Institute and the New York University Medical School. A total of 58 male Long-Evans hooded rats (206–528 g at recording) obtained from Charles River Laboratories were used as subjects. Rats were singly housed in polypropylene cages and maintained on a 12 h light/dark schedule. They were given *ad libitum* access to food and water except during the behavioral procedure, when access to water was given twice a day (30 min in the experimental chamber and 30 min in the home cage). Subjects were handled (5 min per day) and weighed daily to assess their adaptation to water restriction. Behavioral training and electrophysiological recordings were made during the light phase of the 12 h light/dark cycle.

Behavioral procedure. The behavioral procedure has been detailed previously[3]. Odor discrimination ability was assessed with a two-alternative forced-choice odor discrimination task for water reward. Rats were trained in sessions of 30 min, 5 d per week. The behavioral apparatus consisted of a plastic box (30 × 30 × 40 cm; Vulintus) containing a central odor port on one wall and two opposite water ports on the left and on the right walls (**Fig. 1c**). Rats initiated a trial by poking their nose in the odor sampling port to initiate odor onset; the rat was required to hold in the odor port for at least 350 ms for trial initiation. Depending on odor identity, the rat then had to make a choice of a left or right reward port within 3 s to initiate water delivery. Odor presentations were randomized and at least 25 trials with each stimulus were included in each test. Rats usually performed about 100 trials in the 30-min sessions. Mean error rate within a session was used as the measure of discrimination and compared across sessions with ANOVA for repeated measures and Student's *t* test for paired samples.

Pre-training. During the pretraining phase, the rats learned to perform the task with an easily discriminable pair of monomolecular odorants (vanilla versus peppermint) until criterion performance (error rate <0.25 for both sides) was attained during three successive sessions. On average, rats were able to reach this criterion after (mean ± s.d.) 15 ± 3 d of training; the rats showing this performance belonged to the conditioned group ($n = 39$ rats). Some rats ($n = 8$) failed to acquire proficiency at this same task after 25 d of training (error rate never below <0.4) and were allocated to the pseudo-conditioned group.

Mixtures discrimination. Mixtures were created by adding odorant components to mineral oil in amounts that provided identical component concentrations (approximately 100 p.p.m.) within the mixture on the basis of individual odorant vapor pressure[3]. The initial ten-component mixture (10c) included the following monomolecular odorants (vapor pressure in mm Hg indicated in parentheses): isoamyl acetate (5.00), nonane (4.29), ethyl valerate (4.80), 5-methyl-2-hexanone (4.60), isopropylbenzene (4.58), 1-pentanol (6.11), 1,7- octadiene (6.15), 2-heptanone (3.86), heptanal (3.52) and 4-methyl-3-penten-2-one (6.69). The 10c mixture was then degraded by the removal of one (isoamyl acetate) or two (isoamyl acetate and nonane) components (10c–1, 10c–2) or transformed by the replacement of one component (isoamyl acetate) by another component (limonene (1.98)) (10cR1). During the test phase, the rat's ability to discriminate the 10c core mixture from its subsets was evaluated throughout a short (2 d) or a longer training (8 d).

Recording and odorant stimulation. The electrophysiological recordings in olfactory bulb and the aPCX were performed in different rats. There were no differences in learned behavior between rats randomly chosen for olfactory bulb recordings or for aPCX recordings. Details of single-unit recording and odorant-response characterization techniques for layer II/III anterior piriform cortex neurons and for mitral/tufted cells have been reported elsewhere[10,19]. Two categories of rats were recorded: one was never trained nor exposed to odors (naive group, $n = 9$ rats), whereas the other was trained in the odor discriminative task described above (conditioned ($n = 32$ rats) and pseudo-conditioned ($n = 8$ rats) groups). Electrophysiological recordings were always performed the day after the end of the training. All the rats had had access to water during the 20 h before recording. Rats were anesthetized with urethane (1.5 g per kilogram body weight) and were freely breathing with the respiratory cycle monitored through a piezoelectric sensor strapped to the chest. Single units (filtered at 300 Hz to 3 kHz) and LFPs (filtered at 0.1–300 Hz) were recorded simultaneously with a single tungsten microelectrode (1–5 MΩ). Signals were digitized at 10 kHz with a CED micro1401 and analyzed with Spike2 (CED). Mitral/tufted cells were identified by antidromic stimulation of the lateral olfactory tract. Layer II/III anterior piriform cortex neurons were identified by lateral olfactory tract–evoked responses and histological confirmation.

Odorants were delivered with a flow-dilution olfactometer, with a constant, 1 l min^{-1} flow of filtered air presented 1–2 cm from the rat's nose. Saturated odorant vapor was added at 0.1 l min^{-1} to the clean air stream by means of computer controlled solenoids to produce an approximate dilution of 1:10 of saturated vapor. Odorant stimulus onset was triggered off the respiratory cycle to coincide with the transition from inhalation to exhalation. The stimulus duration was 2 s with at least 30 s interstimulus intervals to reduce cortical adaptation. All the odor stimulations were presented during the fast-wave states of anesthesia because the single units showed reduced responsiveness to odors during the slow-waves periods[47]. Each unit was tested with each stimulus presented 3–5 times randomly. The stimuli used during recordings were the same odorants with the same concentration as those used for the behavioral task.

Data analysis. Electrophysiological data were analyzed as previously described[10,48]. Neural responses to odors were assessed at the single-unit level, at the ensemble of neurons level and at the whole neural network level through the examination of odor-evoked oscillatory activities. Single-unit spike sorting, cluster cutting, waveform analysis and fast Fourier transform power analyses of the local field potentials were all performed in Spike2.

The single-unit nature of the recordings was verified by at least a 2-ms refractory period in interval histograms. Single-unit responses to odors were analyzed with peri-stimulus histograms: the response magnitudes of mitral cells and aPCX neurons to odorants were quantified as the difference in number of spikes evoked during the 0–3 s after stimulus onset compared with a 3 s period before the stimulus. Odor-responsive neurons were defined by firing rates with a 30% odor-evoked increase above spontaneous activity. Suppression indicates a 30% or more decrease in odor-evoked activity to the baseline.

The breadth of tuning metric[49] was calculated to assess the proportional distribution of a cell's response across the four complex stimuli used in this study (10c, 10c–1, 10c–2 and 10cR1). The entropy coefficient was calculated as follows:

$$H = -K\Sigma_i p_i \log p_i$$

where H is the breadth of tuning, K is the scaling constant ($K = 1.661$ for four stimuli, set so that $H = 1$ when the neuron responds equally well to all stimuli) and p_i is the proportion of the response to stimulus i relative to the summed response to all four stimuli. The entropy measure varies from 0 for a cell responding exclusively to one stimulus (that is, narrowly tuned) to 1 for a cell responding equally to all of the four stimuli (that is, broadly tuned). To examine differences in breadth of tuning between naive and trained rats, Student's *t* tests for unpaired samples were performed on the entropy values.

Virtual ensemble data[10] were created from combined single-unit recordings across rats. Pearson correlation coefficients (r) were calculated for responses across stimulus pairs for aPCX neurons and mitral cells to examine whether the populations of neurons encoded the10c initial mixture similarly to (significant correlation, assessed using the Fisher's r to Z test) or differently from (non-significant correlation) its morphed versions (10c–1, 10c–2, 10cR1). The stability of odor responses was verified through the significant correlation between multiple repetitions of 10c. Statistical comparisons between the 10c self correlation and the correlations obtained with its morphed versions were then assessed with the test of the difference between correlation coefficients[50].

Odor-evoked oscillatory activities were estimated in the beta (15–35 Hz) and gamma (40–80 Hz) frequency bands. For each olfactory stimulation, power spectra (fast Fourier transform size, 0.2048 s; Hanning window) were calculated for the 0–3 s period after stimulus onset and normalized by the power of the 3-s pre-stimulus baseline for comparisons between rats and experimental conditions. As the distribution of power value is not normal, statistical comparisons between naive and trained rats were performed using the Mann-Whitney U test for unmatched samples.

Histology. After recording, rats were overdosed with anesthetic and transcardially perfused with saline and 4% (wt/vol) paraformaldehyde. Coronal brain sections (40 µm thick) were cut and stained with cresyl violet for determination of electrode positions (**Supplementary Fig. 1a**).

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47. Wilson, D.A. Single-unit activity in piriform cortex during slow-wave state is shaped by recent odor experience. *J. Neurosci.* **30**, 1760–1765 (2010).
48. Fletcher, M.L., Smith, A.M., Best, A.R. & Wilson, D.A. High-frequency oscillations are not necessary for simple olfactory discriminations in young rats. *J. Neurosci.* **25**, 792–798 (2005).
49. Smith, D.V. & Travers, J.B. A metric for the breadth of tuning of gustatory neurons. *Chem. Senses* **4**, 215–229 (1979).
50. Preacher, K.J. Calculation for the test of the difference between two independent correlation coefficients. <http://quantpsy.org/> (2002).