NN-A24405

SUPPLEMENTARY INFORMATION
for
OLFACTORY PERCEPTUAL STABILITY AND DISCRIMINATION

Dylan C. Barnes[1]*, Rylon D. Hofacer[1]*, Ashiq R. Zaman[1], Robert L. Rennaker[2] and Donald A. Wilson[1,3,4]

[1]Neurobehavioral Institute

Department of Zoology

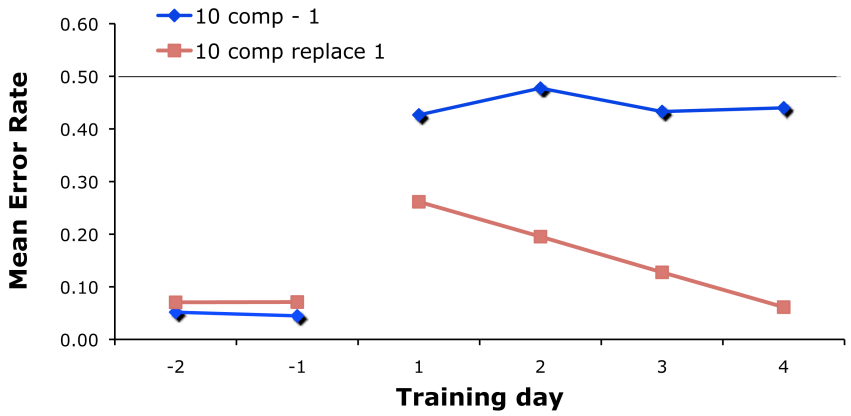
University of Oklahoma

[2]School of Aerospace and Mechanical Engineering

University of Oklahoma

Nature Neuroscience: doi:10.1038/nn.2217

Animals in the 10c vs 10c replace 1 condition were already different from chance by the end of the first session.



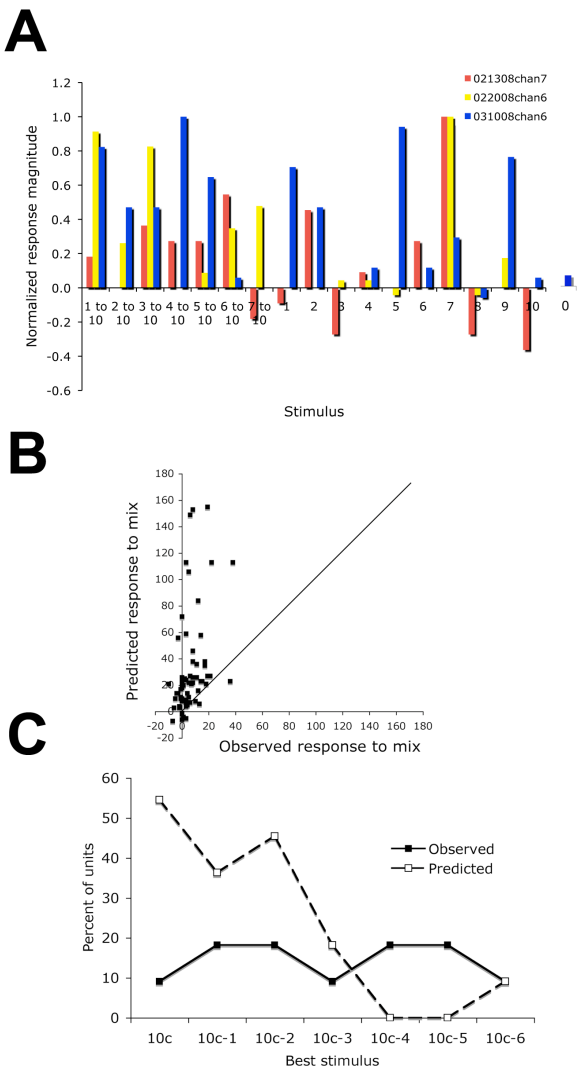
Supplement Figure 1. Representative examples of behavioral odor discrimination performance between complex mixtures with one missing component or one replaced component. Animals were trained on a variety of odor discrimination tasks, including vanilla detection (1:1000 dilution), performing at greater than 90% accuracy. Performance in odor mixture discrimination matched that predicted based on cortical ensemble performance.

Odorant mixture morphs

The odorants and their properties used in the described experiments are listed here. The primary data used odorants **A** through **J** as the initial mixture, with each component at approximately 100 PPM. Removal and replacement protocols are as shown in manuscript Figure 1. Thus, the 10-component mixture with one component removed included odorants **B** through **J**. The 10-component mixture with one component replaced had isoamyl acetate replaced with 3-methyl-2-buten-1-ol. When two components were removed, the removed components were isoamyl acetate and nonane. When two components were replaced, isoamyl acetate was replaced with 3-methyl-2-buten-1-ol and nonane was replaced with propyl butyrate (**A** – **A'** and **B** – **B'**).

observed distributions of best stimuli were significantly different from each other (Chi-Square = 78.8, df = 6, $p < 0.001$).

As shown in Supplement Figure 2, cortical unit responses to complex mixtures are strongly dominated by mixture suppression effects, as judged from a comparison with the predicted response based on summation of responses to the components. The mixture suppression may in part be due to non-independence of cortical afferent activity evoked by different odorants due to overlapping activation of broadly tuned receptors [21] and/or due to inhibition within the olfactory pathway [22]. While mixture suppression has been previously reported for binary mixtures in piriform cortex [20], the present results suggest one consequence of this suppression is to help distribute encoding of different mixture combinations across a broader ensemble of neurons than would occur if responses added linearly. The data suggest that without mixture suppression, representations become dominated by larger mixtures with relatively few cells maximally responsive to smaller mixtures.



Supplement Figure 2. Observed single-unit response magnitude to odor mixtures did not correspond to that predicted based on

graded response change as mixture component membership changed. Although not precisely sigmoid in shape as might be predicted based on hippocampal results [12], when combined with the behavioral discrimination data, there are clearly both pattern completion and pattern separation components. Perhaps more subtle shifts in stimulus components than are possible with 10-component mixtures are required to fully detect such a relationship. Nonetheless, at the behavioral level an abrupt shift between completion and separation occurred as the stimulus mixture morphed from a 90% to 80% overlap, and this corresponded to a relatively large de-correlation between piriform cortical ensemble activity within this same region.

Supplement References

1. Derby, C.D., Girardot, M.N. & Daniel, P.C. Responses of olfactory receptor cells of spiny lobsters to binary mixtures. II. Pattern mixture interactions. *J Neurophysiol* **66**, 131-139 (1991).
2. Bell, G.A., Laing, D.G. & Panhuber, H. Odour mixture suppression: evidence for a peripheral mechanism in human and rat. *Brain Res* **426**, 8-18 (1987).
3. Lin, D.Y., Shea, S.D. & Katz, L.C. Representation of natural stimuli in the rodent main olfactory bulb. *Neuron* **50**, 937-949 (2006).
4. Tabor, R., Yaksi, E., Weislogel, J. & Friedrich, R.W. Processing of odor mixtures in the zebrafish olfactory bulb. *The Journal of Neuroscience* (2004).
5. Giraudet, P., Berthommier, F. & Chaput, M. Mitral cell temporal response patterns evoked by odor mixtures in the rat olfactory bulb. *J Neurophysiol* **88**, 829-838 (2002).
6. Khan, A.G., Thattai, M. & Bhalla, U.S. Odor representations in the rat olfactory bulb change smoothly with morphing stimuli. *Neuron* **57**, 571-585 (2008).
7. Wilson, D.A. Comparison of odor receptive field plasticity in the rat olfactory bulb and anterior piriform cortex. *J Neurophysiol* **84**, 3036-3042 (2000).
8. Lei, H., Mooney, R. & Katz, L.C. Synaptic integration of olfactory information in mouse anterior olfactory nucleus. *J Neurosci* **26**, 12023-12032 (2006).
9. Scott, J.W., McBride, R.L. & Schneider, S.P. The organization of projections from the olfactory bulb to the piriform cortex and olfactory tubercle in the rat. *J Comp Neurol* **194**, 519-534 (1980).
10. Buonviso, N., Revial, M.F. & Jourdan, F. The Projections of Mitral Cells from Small Local Regions of the Olfactory Bulb: An Anterograde Tracing Study Using PHA-L (Phaseolus vulgaris Leucoagglutinin). *Eur J Neurosci* **3**, 493-500 (1991).
11. Johnson, D.M., Illig, K.R., Behan, M. & Haberly, L.B. New features of connectivity in piriform cortex visualized by intracellular injection of pyramidal cells suggest that "primary" olfactory cortex functions like "association" cortex in other sensory systems. *J Neurosci* **20**, 6974-6982 (2000).
12. McClelland, J.L. & Goddard, N.H. Considerations arising from a complementary learning systems perspective on hippocampus and neocortex. *Hippocampus* **6**, 654-665 (1996).
13. Lynch, G. *Synapses, circuits and the beginnings of memory* (MIT Press, Cambridge, MA, 1986).
14. Wilson, D.A. Rapid, experience-induced enhancement in odorant discrimination by anterior piriform cortex neurons. *J Neurophysiol* **90**, 65-72 (2003).
15. Zou, Z. & Buck, L.B. Combinatorial effects of odorant mixes in olfactory cortex. *Science* **311**, 1477-1481 (2006).
16. Wilson, D.A. Habituation of odor responses in the rat anterior piriform cortex. *J Neurophysiol* **79**, 1425-1440 (1998).
17. 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).
18. Cometto-Muniz, J.E., Cain, W.S. & Abraham, M.H. Quantification of chemical vapors in chemosensory research. *Chem Senses* **28**, 467-477 (2003).
19. Kadohisa, M. & Wilson, D.A. Separate encoding of identity and similarity of complex familiar odors in piriform cortex. *Proc Natl Acad Sci U S A* **103**, 15206-15211 (2006).
20. Kadohisa, M. & Wilson, D.A. Olfactory cortical adaptation facilitates detection of odors against background. *J Neurophysiol* **95**, 1888-1896 (2006).
21. Malnic, B., Hirono, J., Sato, T. & Buck, L.B. Combinatorial receptor codes for odors. *Cell* **96**, 713-723 (1999).
22. Isaacson, J.S. & Strowbridge, B.W. Olfactory reciprocal synapses: dendritic signaling in the CNS. *Neuron* **20**, 749-761 (1998).

23. 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).
24. Rennaker, R.L., Chen, C.F., Ruyle, A.M., Sloan, A.M. & Wilson, D.A. Spatial and temporal distribution of odorant-evoked activity in the piriform cortex. *J Neurosci* **27**, 1534-1542 (2007).
25. Kay, L.M., Crk, T. & Thorngate, J. A redefinition of odor mixture quality. *Behav Neurosci* **119**, 726-733 (2005).
26. Kay, L.M., Lowry, C.A. & Jacobs, H.A. Receptor contributions to configural and elemental odor mixture perception. *Behav Neurosci* **117**, 1108-1114 (2003).
27. Jinks, A. & Laing, D.G. The analysis of odor mixtures by humans: evidence for a configurational process. *Physiol Behav* **72**, 51-63 (2001).
28. Linster, C. & Smith, B.H. Generalization between binary odor mixtures and their components in the rat. *Physiol Behav* **66**, 701-707 (1999).
29. Staubli, U., Fraser, D., Faraday, R. & Lynch, G. Olfaction and the "data" memory system in rats. *Behav Neurosci* **101**, 757-765 (1987).

TABLE 1

Standard Mixture and Morphs for ensemble and behavioral analyses

	Odorant	Vapor pressure (mm Hg)	Concentration at sea level (PPM)	Dilution in µL/10 mL mineral oil for 100 PPM*	Dilution in µL/10 mL mineral oil for 350 PPM*	Quality descriptor
A	Isoamyl acetate	5.00	6579	152	532	Fruity, banana
B	Nonane	4.29	5645	177	620	Gasoline
C	Ethyl valerate	4.80	6316	158	554	Fruity, apple
D	5-methyl-2-hexanone	4.60	6053	165	578	Fruity, sweet
E	Isopropylbenzene	4.58	6026	166	581	Aromatic
F	1-pentanol	6.11	8039	124	435	Fusel, sweet
G	1,7-octadiene	6.15	8092	124	433	
H	2-heptanone	3.86	5079	197	689	Fruity, spicy
I	Heptanal	3.52	4632	216	756	Green, wine
J	4-methyl-3-penten-2-one	6.69	8803	114	398	Pungent, vegetable
	REPLACEMENT					
A'	3-methyl-2-buten-1-ol	6.90	9079	110	386	Fruity, green
B'	Propyl butyrate	5.95	7829	128	447	Fruity, pineapple
C'	limonene	1.98	2605	384	1343	Herbaceous, mint

- Approximate concentration assuming 1:1 airflow dilution in clean air

In order to ensure the observed effects were not limited to these specific odorant combinations, additional aPCX ensemble recordings were made with different morphs from the standard in Table 1 and/or at a higher concentration. Thus, in one set (n=13 units) the 10-component mixture with one removed was missing component **J**, 4-methyl-3-penten-2-one, and the 10-component mixture with one replaced had 4-methyl-3-penten-2-one replaced with 3-methyl-2-buten-1-ol (**J** replaced with **A'**). Component concentration was approximately 350 PPM. In another set (n = 14 units), removal and replacement were as described in Table 1, but the concentration was approximately 350 PPM. As shown in text Figure 2, ensemble data were consistent across all morph protocols.

In the behavioral experiments, in addition to the mixtures shown in Table 1, several variations were tested as listed in Table 2, along with the proportion of animals that attained criterion within 2 days of training.

TABLE 2

Variations in morphs tested in behavioral assays

discriminate	% reaching criterion by day 2		
10c vs 10c	**0.00**	n = 4	
			missing odor (listed in order tested)
10c vs 10c-1	0.13	n = 8	*isoamyl acetate*
10c vs 10c-1	0.25	n = 4	*ethyl valerate*
10c vs 10c-1	0.50	n = 4	*isopropylbenzene*
10c vs 10c-1	0.75	n = 4	*1,7,octadiene*
Mean 10c-1	***0.41***		
10c vs 10c-2	**1.00**	n = 4	*isoamyl acetate and nonane*
10c vs 10c-3	**1.00**	n = 4	*isoamyl acetate, nonane and ethyl valerate*
			replace (tested random order)
10c vs 10cR1	0.75	n = 4	*isoamyl acetate with 3-methyl-2-buten-1-ol*
10c vs 10cR1	1.00	n = 4	*isoamyl acetate with propyl butyrate*
10c vs 10cR1	0.75	n = 4	*isoamyl acetate with limonene*
10c vs 10cR1	0.50	n = 4	*4-methyl-3-penten-2-one with limonene*
Mean 10cR1	***0.75***		

Sensory discrimination is dependent on separation of input patterns of activity evoked by different stimuli, while perceptual stability in the face of minor variation is dependent on memory-based completion of degraded or noisy inputs. Here, we looked for evidence of pattern separation and completion in the olfactory system. Stimuli consisted of complex, 10 component mixtures that were morphed by either removing a standard set of components, or replacing standard components with novel ones. The results showed that anterior piriform cortex single-unit ensembles showed a consistent change in response patterns as individual standard components were removed. However, the addition of a single unusual component to the complex mix resulted in a dramatic shift to pattern separation. In contrast, mitral cell ensembles in the olfactory bulb, the afferent to the anterior piriform cortex, showed stable pattern separation across all morphs. Behavioral discrimination performance matched that of the anterior piriform cortical ensembles. Removal of a single component was difficult and replacement of a single component was easy to discrimination from the standard 10 component mixture.

Interactions between mixtures of odorants can occur throughout the olfactory pathway, including olfactory sensory neurons [1, 2], glomeruli (which are the sensory neuron target in the olfactory bulb)[3], and local olfactory bulb circuits [4-7]. Several recent studies suggest that activity of both olfactory bulb glomeruli and olfactory bulb projection neurons (mitral/tufted cells) reflects either the presence of an individual odorant feature [7, 8], and/or reflects interactions between individual components [4, 6]. Thus, it appears that the pattern of olfactory bulb output to the olfactory cortex primarily reflects information about what component features are present in the inhaled sample, along with some interactions between those components.

In contrast to the olfactory bulb, the olfactory cortex is anatomically organized as an auto-associative, combinatorial array, receiving information about odorant feature input via distributed and overlapping olfactory bulb projections, which allows direct convergence of multiple feature information [9, 10]. The combinatorial properties are enhanced through an extensive intracortical association fiber system [11], which shows robust synaptic plasticity hypothesized to be critical for optimal pattern separation/completion characteristics [12]. This circuitry is broadly similar to that of the hippocampal formation [13]. In contrast to single units within the olfactory bulb, olfactory cortical units (e.g., anterior olfactory nucleus [8] and piriform cortex [14, 15]) respond to odorant mixtures distinctly from their components, consistent with configural, odor object-oriented encoding.

The present experiments attempted to identify evidence of pattern separation and completion at either the cortical or behavioral level in response to complex odorant mixtures. The results suggest that piriform cortical ensembles are capable of both pattern separation and completion, and that this ensemble activity predicts behavioral perceptual performance.

Nature Neuroscience: doi:10.1038/nn.2217

METHODS

Subjects

Male Long-Evans hooded rats (50~400g at recording), obtained from Harlan Lab Animals, were used as subjects. Food and water were available ad lib. Animal care and use conformed to NIH guidelines and were in accordance with the University of Oklahoma IACUC.

Recording and odorant stimulation

Details of single-unit recording and odorant-response characterization techniques for layer II/III anterior piriform cortex neurons[16] and of mitral/tufted cells[17] have been reported in detail elsewhere. Briefly, animals were anesthetized with urethane (1.5 g/kg) and were freely breathing with the respiratory cycle monitored through a piezoelectric device on the chestwall. The single-unit nature of the recordings was verified by at least a 2-ms refractory period in interval histograms and stable waveforms. Mitral/tufted cells were identified by antidromic stimulation of the lateral olfactory tract. Most mitral/tufted cell recordings were made near the midline of the dorsal surface of the olfactory bulb. Layer II/III anterior piriform cortex neurons were identified by lateral olfactory tract-evoked responses and/or histological confirmation. After isolation of a single-unit, 2 sec odor stimuli were delivered for each odorant, with stimulus order randomized and at least 60 sec inter-stimulus intervals. Odorant stimulus onset was triggered off the respiratory cycle to coincide with the transition from inhalation to exhalation. Each stimulus was repeated between 3 and 10 times for each cell. To be included the cells had to show at least a minimal response (50% change in mean firing rate) in response to at least one of the odorants. In most cases no more than 2-3 units were recorded from a given animal because of our past work showing dramatic changes in cortical response over prolonged stimulation protocols. An individual cell was tested with only one stimulus mixture condition. In order to confirm that the findings with this original stimulus set were reliable with different stimulus manipulations, smaller sets of different cells were tested with different stimulus sets.

Odorants were delivered with an olfactometer, with a constant, 1 liter per minute flow of charcoal-filtered, humidified air presented 1–2 cm from the animal's nose. Odorants were diluted in mineral oil to the desired concentration (approximately 100 or 350 PPM) based on individual odorant vapor pressure. Mixtures were created by adding odorant components to mineral oil in amounts that provided identical component concentrations within the mixture. Mixtures consisted of from four to ten components, with both number and identity of components varied within and across experiments. Component monomolecular odorants (and vapor pressure) included: isoamyl acetate (5.00 mm Hg), nonane (4.29),

Nature Neuroscience: doi:10.1038/nn.2217

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), 4-methyl-3-penten-2-one (6.69), 3-methyl-2-buten-1-ol (6.90), propyl butyrate (5.95) and limonene (1.98). Approximate concentration in PPM of saturated vapor was calculated as ((vapor pressure in mm Hg/760 mm Hg)*1,000,000), and dilutions obtained by mixing with mineral oil. See Cometto-Munez et al., [18] for a discussion of caveats in the use of these calculations. Molecular structure and spatial patterns of odorant-evoked olfactory bulb glomerular layer activity for each of these stimuli can be found online at http://leonserver.bio.uci.edu/index.jsp.

Electrophysiological data analysis

Mitral/tufted cell and piriform cortical responses to odors were assessed at both the single-unit and ensemble levels. Virtual ensemble data were created from combined single-unit recordings across animals, as previously described [19]. Single-unit responses to odors were analyzed with peri-stimulus histograms. Response magnitudes were calculated from summed spike counts during stimulation with baseline activity (3 sec pre-stimulus) subtracted. Specific analytical detail is provided in the Results.

Histology

Following recording, animals were overdosed with anesthetic, transcardially perfused with saline and 4% paraformaldehyde, and the brains subsequently sectioned coronally at 40 μm, and stained with cresyl violet for determination of electrode positions.

Animal training

Odor discrimination ability was assessed in four rats different than those used for recording. Animals were given daily limited access to water and trained in a two-alternative forced choice task for water reward. A nose poke into the center, odor delivery port was monitored with an infrared photocell and initiated odor onset at a variable latency of 0-300 ms. Depending on the odor identity, the rat then had to make a choice of a left or right water reward port within 3 sec to initiate water delivery. Odorants were identical in quality and concentration to those used in the electrophysiological recordings. Training sessions occurred at least 5 days/week and lasted at least 30 min. Animals initiated a mean of 159 ± 9 trials within individual sessions. Discrimination testing for specific odor pairs lasted at least four consecutive days for the data shown in text Figure 1. Animals were trained at least 2 days for the data shown in Figure 2. Mean error rate within a session was used as the measure of discrimination and compared across odor tasks with analysis of variance. An example of daily performance is shown in Supplement Figure 1.

It should be noted that stimulus intensity (odor concentration) was not constant in the stimulus sets used here. Although the individual components within a mixture were of the same concentration, the concentration of the mixture could vary as a function of number of components. However, an examination of the observed findings reported here, from additivity to discriminability, suggests that none can be accounted for by intensity variation, and in fact are generally counter to an intensity-based explanation. For example, although the mixtures 10c and 10cR1 have the same concentration they are much easier to discriminate at the ensemble and behavioral levels than 10c and 10c-1 which differ in total concentration.

It should also be noted that removal of some components, although all were matched for concentration, was easier to detect than others. For example, most animals discriminated the 10C mix from the 10c mix when 1,7,octadiene was removed, but not when other individual components were removed. Nonetheless, discrimination of replacement was significantly easier than discrimination of a single removal, as described in the main text.

ADDITIONAL RESULTS

Supplement Fig. 2A shows normalized response magnitudes of three different cells to both the mixtures and their components. Again, cells responded to the mixtures independently, with the response of a given cell to one mixture unpredictable from how it responded to similar mixtures. In those cells tested with both mixtures and components, we did not find any cells that responded to the mixtures without responding to at least one component. In a small set of cells (n = 11), both responses to the mixtures and responses to the individual components were examined. Observed response magnitude (evoked spike count 0-3 sec post-stimulus onset minus spontaneous spike count 3 sec prior to stimulus onset) to the mixtures (varying from 10 components to 4 components) were compared with response magnitudes predicted from algebraic summation of responses to all of the individual components (Supplement Fig. 2B). The predicted response magnitude was consistently and significantly higher than the observed response magnitude (paired t-test, $t(76) = 5.92$, $p < 0.001$), suggesting either considerable mixture suppression, as has previously been reported in piriform cortex [20] and/or substantial non-independence of input driven by different monomolecular odorants. We also examined the observed and predicted "best" stimulus for each of these cells, defined as that stimulus that evoked the largest change in spike count. As can be seen in Supplement Figure 2C, the predicted best stimulus for most cells were those mixtures containing the largest number of components, with some of the smaller mixtures not represented as the best stimulus for any cell. However, in the observed data, the observed best stimulus varied between cells, with a nearly equal representation of each mixture across this population of cells. The predicted and

addition of responses to the components. (**A**) Cells were tested with both mixtures (4-10 components) and with individual components. Response examples from three representative cells are shown. (**B**) Predicted response magnitudes (driven spike count) to the mixtures based on component responses are plotted against the observed response to that mixture. There was a strong mixture suppression effect in the observed responses compared to predicted responses. (**C**) Proportion of observed and predicted cells with maximal response magnitudes to each odor mixture. Predicted cell responses are based on summed component responses, and show a significant skew toward over-representation of more complex mixtures. Observed cells, which have mixture suppression effects in their responses, show relatively evenly distributed representation of all mixtures. Values do not sum to 100% because some cells responding equally well to more than one odor.

CONCLUSIONS

The present data make several novel observations concerning encoding of complex odorant mixtures in the anterior piriform cortex. First, single cortical units respond to odor mixtures in a discrete manner, not directly predictable from responses to the components, nor directly predictable from the responses of the same cell to mixtures with substantially overlapping (e.g., 90%) components. These mixture responses presumably reflect unique combinations of convergent afferent and association fiber inputs to individual cortical neurons, as predicted for a combinatorial circuit of this type [23]. Together with previous work [14, 24], these data suggest that odor mixtures can be treated as unique odor objects by cortical neurons, with encoding of a particular odor distributed across intermingled cells across large regions of the piriform cortex. The present results also add to the growing behavioral evidence for configural perception of odor mixtures [25-29].

Second, component replacement had significantly greater impact on both cortical ensemble and perceptual outcome than component removal. It is hypothesized that inclusion of a low probability component into a familiar mixture may be of more ecological significance, and thus more likely to drive separation, than the loss of a component from such a mixture. We propose that the addition of a novel component (replacement), while discriminable, may modify the percept of the entire odor. Thus, behavioral identification of the specific new component may not be possible, but rather the entire percept may change [29]. It is suggested that examination of pattern separation and completion in other systems should be extended to look for similar distinctions in the effects of different protocols of stimulus morphing.

The third novel finding, and perhaps of greatest general importance is that, in contrast to the unpredictable response of cortical single units to morphing mixtures, cortical ensemble activity showed a