Encoding of mixtures in a simple olfactory system

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SUMMARY

Natural odors are usually mixtures; yet humans and animals can experience them as unitary percepts. Olfaction also enables stimulus categorization and generalization. We addressed how these computations are performed with the responses of 168 locust antennal lobe projection neurons (PNs) to varying mixtures of two monomolecular odors, and 174 PNs and 209 mushroom body Kenyon cells (KCs) to mixtures of up to 8 monomolecular odors. Single PN responses showed strong hypo-additivity, and population trajectories clustered by odor concentration and mixture similarity. KC responses were much sparser on average than with PNs and often signaled the presence of single components in mixtures. Linear classifiers could read out the responses of both populations in single time bins to perform odor identification, categorization, and generalization. Our results suggest that odor representations in the mushroom body may result from competing optimization constraints to facilitate memorization (sparseness) while enabling identification, classification and generalization.

INTRODUCTION

Key computational problems of olfaction include discrimination (Abraham, 2004; Linster et al., 2002; Lu and Slotnick, 1998; Rubin and Katz, 1999; Uchida and Mainen, 2003), concentration invariance (Bhagavan and Smith, 1997; Stopfer et al., 2003; Uchida and Mainen, 2007) categorization (grouping of stimuli by shared features), generalization (assignment of novel stimuli to a group, based on shared features), and segmentation (of components from a mixture, or of signal from background) (Mainen, 2006; Wang et al., 1990; Wilson and Mainen, 2006). These object recognition problems (DiCarlo and Cox, 2007) are not specific to olfaction but they are interesting to study there, because olfactory systems solve them in very few neural steps. Using locusts as models, we gained some understanding of the representation formats for simple odors in the first three relays of its olfactory system—the antennal lobe (AL), mushroom body (MB) and beta lobe (bL)—and of the computations carried out by these circuits (Cassenaer and Laurent, 2007; Mazor and Laurent, 2005; Perez-Orive et al., 2002; Stopfer et al., 2003). We also discovered that odors at different concentrations generate families (low-dimensional manifolds) of spatio-temporal representations (Stopfer et al., 2003), providing a neural substrate for concentration invariance. In this study, we turn to odor mixtures. Most natural odors comprise many
components, usually mixed in particular ratios. Mixtures can be perceived as wholes ("coffee", "grapefruit") (Jinks and Laing, 1999), but they can also be classified into categories, with various degrees of refinement ("fruity" → "citrusy" → "grapefruit"). Humans can typically identify no more than ~3 components, but sometimes as many as 8–12 familiar components in a blend (Jinks and Laing, 1999) and insects and rodents can likely do better (Hurst and Beynon, 2004; Reinhart et al., 2010). Also, natural odors such as floral scents can vary from one flower to the next, or from one time of the day to another (Wright and Thomson, 2005). For foraging insects, this necessitates that animals be able to identify individual flowers (to prevent costly repeated visits), and that they generalize (so as to sample flowers of the same variety, species or type) (Reinhart et al., 2010; Wright et al., 2008; Wright and Thomson, 2005). How does the brain solve both discrimination and generalization problems? Our goal was to find out, using the locust system, whether and how the formats of representations for odors support these computations. We begin with binary mixtures (Fig. 1A–D, Methods), and then expand to multi-component mixtures with a set of eight monomolecular odors, paraffin oil (their dilution substrate) and 32 of the 211 possible mixtures of two, three, four, five and eight of those odors (44 stimuli in all, see Fig. 1E, Methods). We recorded from 342 projection neurons (PNs, the analog of vertebrate mitral cells) and 209 Kenyon cells (KCs, the mushroom body neurons) in 61 animals.

RESULTS

Our primary data are neural responses to odor mixtures of up to 8 pure components. Unless otherwise noted, the response window is defined as the one-second period from 0.1 s to 1.1 s following odor onset (the 0.1 s offset is to account mostly for stimulus delays external to the animal), and the baseline window is the one-second period from −1.1 s to −0.1 s before odor onset.

Representations of Binary Mixtures by Single PNs

We first examined the responses of single PNs to binary mixtures of octanol and citral. Figures 2A–D show the response of a sample PN to the mixtures tested. The responses are mixture specific, reliable, and temporally patterned, as previously observed for PN odor responses (Perez-Orive et al., 2002; Stopfer et al., 2003).

We examined the extent to which mixture responses (of single PNs) could be explained by component responses. Figure 2C illustrates that mixture responses can deviate from the sums of the corresponding component responses. We therefore tested how well weighted sums of the components

\[ \text{fit}(t) = a \text{citral}(t) + b \text{octanol}(t) + c, \]

describe mixture responses. The citral and octanol response functions [citral(t) and octanol(t)] were expressed as mean firing rates across trials in consecutive 50 ms bins, in response to the concentrations present in the mixture. We tested the following models, which differ by the constraints on the fit coefficients, allowing the component responses a temporal jitter of up to three time bins:

<table>
<thead>
<tr>
<th>Model</th>
<th>Constraint</th>
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<tr>
<td>Constant</td>
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We fit all mixture responses of every PN to each one of these models and used Bayesian model selection to select among them (see Methods and Supplementary Information for details).

Figure 2E shows some fits for the response of the PN in Fig. 2A–D to the mixture cit140:oct120. The mixture response is in black; component responses are red (oct120) and green (cit140). The selected fit was a scaled and lagged version of the citral response (*, $R^2 = 0.49$). The better fit ($R^2 = 0.51$) of the free mixture model did not justify its increased complexity.

We summarize the results of this fitting procedure over the population in Fig. 2F (see also SI). The three matrices in Fig. 2F are mixtures x PNs. Matrix cells are colored according to the coefficients (≠0) in the fit: red for octanol, green for citral, yellow for both, black for neither (i.e., constant model). We sorted the cells by their preference for responses of one type or another, and then by their center of gravity within each group. The matrix on the left shows cells the majority of whose responses were best fit using pure citral responses; the second matrix shows the same with octanol. The small matrix on the right shows cells the majority of whose responses were best fit using both component responses, or for which no component response dominated. Not shown are 24 PNs for which all mixture responses were fit best by a constant model.

Most fits were of the citral or octanol type i.e., the mixture response was best fit by scaling the response to one component. Varying degrees of concentration sensitivity were observed: some PNs responded at all dilutions of their “dominant” component; others did so only above a particular concentration (specific to that PN and odor). Such responses would facilitate concentration-invariant and concentration-sensitive computations, respectively.

### Representations of Binary Mixtures by PN Populations

Because odor representations by PNs are highly distributed and varied in time (Fig. 2 and (Laurent and Davidowitz, 1994; Mazor and Laurent, 2005; Stopfer et al., 2003)), because their activity patterns are decoded by individual KCs on which converge many PNs (Jortner et al., 2007) and because KCs have very short effective temporal integration windows (Perez-Orive et al., 2004; Perez-Orive et al., 2002), it is useful to examine PN responses as time-series of instantaneous population vectors, or *trajectories*, and visualize them in an appropriately reduced state space (Broome et al., 2006; Brown et al., 2005; Mazor and Laurent, 2005; Stopfer et al., 2003). Figure 3A illustrates these PN population trajectories in response to octanol (red), citral (green), and their 1:1 mixture (yellow), after dimension reduction by Locally Linear Embedding (LLE) (Roweis and Saul, 2000). The non-linear nature of LLE makes quantitative comparisons between trajectories difficult. Therefore, we also show correlation measures between the trajectories in the full space. The nine matrices in Fig. 3B plot the correlation distance ($D_c$, see Methods) within (matrices along diagonal).
and between the responses (168-PN-vector time series over 3 s) to the three stimuli in Fig. 3A, calculated across different trials with each stimulus. We summarized these matrices by the minimum value of each of the sub-matrices and plotted these values as a matrix of min $D_c$ (Fig. 3C). We then combined these min-$D_c$ matrices with each corresponding set of trajectories (Fig. 3D–F, 5A–C). The combination of correlation measures and LLE trajectories provide both quantitative and qualitative descriptions of the data.

Figure 3D shows the same data as Fig. 3A with the associated min-$D_c$ matrix. The dimension-reduced mixture trajectories (yellow) lie in between those for the two components, an impression supported by intermediate correlation values (inset). Figure 3E represents concentration series for the three stimuli (cit, cit:oct, and oct). Extending previous results (Stopfer et al., 2003), concentration series for 1:1 mixtures, as for single odors, generated families of similar trajectories clustered by odor rather than concentration (see also matrix inset). Finally, we “morphed” one odor into the other in 11 intermediate concentration steps (Fig. 3F). Qualitatively, the population trajectory corresponding to one odor appeared to shift gradually, and impression supported by the correlation matrix inset.

We then directly tested whether the qualitative impressions provided by LLE plots in Fig. 3D–F hold in the full (non-dimension-reduced) space. Fig. 3D suggests that the 1:1 mixture trajectory (yellow) lies almost exactly between those for the two components, an impression supported by intermediate correlation distance values (inset). To confirm this impression in the full space, we computed, for consecutive 100-ms time bins, the projection of the population vector for the mixture onto the plane spanned by the vectors for the two components. Then, we computed the angle between this mixture projection and the time-matched vector for citral, as a fraction of the angle between the simultaneous vectors for the two single odors. This yielded the “projection angle fraction” (PAF) with respect to citral, which is by definition 0 for citral, 1 for octanol, and intermediate for mixtures (values less than 0 are not possible; values greater than 1 are possible, though rare). Figure 3G shows the time course of the mean PAF±S.E.M. (shading) computed over the 10 trials, for ‘mostly citral’ (cit140:30oct, green), 1:1 mixture (140cit:140oct, gold), and ‘mostly octanol’ (30cit:140oct, red), in consecutive 100-ms time bins. During baseline, all three odors had a PAF of ~0.5 (as expected from cells firing randomly and independently at the average baseline rate). At odor onset, however, the PAF for ‘mostly citral’ dropped to ~0.1 while that of ‘mostly octanol’ rose to ~0.9, indicating high similarity to the representations of pure citral and pure octanol, respectively. Confirming the impression from Fig. 3D, the PAF for the 1:1 mixture remained mostly near 0.5 (the low variability indicates that this value was stimulus-driven and not simply due to noise). Nearly 75% of the magnitude of the population vector lay in the projection plane during the early phase of the response (Fig. S3A), and the PAFs computed with respect to octanol were equal to 1 minus the PAFs to citral (Fig. S3B). Hence we conclude that the trajectory for the 1:1 mixture indeed lies almost exactly in between the trajectories for the two components.

Figure 3E suggests that concentration series for single odors and 1:1 mixtures generate families of closely related trajectories (lower-D manifolds), clustered by odor rather than concentration (see matrix inset). We quantified this impression by temporally concatenating the binned population vectors for each trajectory (yielding one large vector for each trajectory for the time window of interest). We then computed Rand indices (Rand, 1971) to measure the agreement between clustering by correlation distance and clustering by odor, or between clustering by correlation distance and clustering by concentration (Fig. 3H; range 0–1; 1=perfect agreement, see Methods). At baseline, both comparisons yielded values close to chance (dashed lines). During the response window, clustering was clearly by odor.
Figure 3F suggests that when one odor is morphed into another, the population trajectory shifts gradually (rather than abruptly, as reported in zebrafish (Niessing and Friedrich, 2010)) towards that for the other odor, passing through their 1:1 mixture trajectory. We quantified this by fitting, in consecutive 100-ms time bins, the correlation distance between each PN vector (full space) for the mixture and that for citral, as a function of the concentration ratio (log_{10} of the ratio of the concentrations in the mixture), to constant, linear, one-, and two-step functions. We then used Bayesian model selection (see Methods) to rank the models at each time by their fit to the data while penalizing them for complexity. In Fig. 3I the time course of the logarithms of the resulting posterior probabilities for each model relative to that for the linear model are shown (mean ± S.E.M. (shading) over trials). At baseline, the constant model is best, indicating no relation between distance and mixture level. Upon odor onset, the linear model quickly dominates and remains superior for most of the response window. Figure S3C shows the results when using fraction octanol as the independent variable, yielding similar results. Figure S3D shows the data and model fits at different times, allowing visual confirmation of the superiority of the linear fit. The superiority of the linear model over the step models suggests that the encoding space defined by PNs is not discretized (at least within the range and resolution of concentrations tested), and allows the spread of odor representations to accommodate fractional changes in the stimulus.

**Representation of Complex Mixtures by Single PNs**

Eight molecules were chosen to be chemically distinct (Fig. 1E) and their concentrations adjusted to evoke electro-antennograms that were reliable, small and comparable in amplitude (Fig. S1C,D), to compensate for differences in vapor pressure or receptor activation and to ensure operation far from saturation. We determined the extent to which the mixture responses of single PNs could be explained by their responses to single components in the mixture. The response of a PN to an n-component mixture was regressed on the constant model (no inputs), and all 2^n - 1 possible combinations of single component responses. For each such input combination, we computed the regression for the unit-, scaled- and free-coefficient models, as well as for lagged versions of these. We then determined the best model using Bayesian model selection (see Methods).

In Fig. 4A we show the response of a sample PN to the mixture ABCD and to the components (left), and some of the fits (right). The selected model in this case was a unit-scaled mixture of odors A, B and C (∗, \(R^2 = 0.68\)). The better fit of a more complex model (the free mixture ‘A and B and C and D’, \(R^2 = 0.75\)) did not justify its increased complexity.

In Fig. 4B we summarize the results of the model fitting procedure over all PNs and all mixtures. The layout is similar to that used for binary mixtures (Fig. 2F), extended to 8 components, and contains nine PN x mixture matrices. Matrix cells are colored according to the component responses used in each fit according to the legend on the right. PNs have been sorted according to the single component response (if any) that explained the majority of their responses, and then by the center-of-mass of their responses within each group. The A, B…Z matrices show the PNs for which a single component dominated. The columns within each matrix have been arranged so that odors containing the component come first followed by those that don’t contain the component, both in order of increasing mixture complexity. The ‘Mixture or Ambiguous’ matrix shows the 6 PNs for which more than one component response was always required to explain the majority of the mixture responses, and the 10 PNs in which no single component response dominated. Not shown are the 34 PNs for which the single components did not provide an adequate fit for any mixture. These data are replotted along with measures of fit quality and further analyses in Fig. S4A–G.
Figure 4B shows that for each of the 8 components, there exist PNs whose responses to mixtures can, in most cases, be fit most conservatively using the single response to that component. Dilution effects are present, with some PNs yielding fits only at the binary mixture level, others up to the 3-mixture level, etc. Such a distribution of response types would allow both concentration-invariant and concentration-sensitive types of olfactory computation.

Representations of Complex Mixtures by PN Populations

The LLE trajectories corresponding to the eight single component odors are shown in Fig. 5A. Consistent with the odors’ distinct chemical composition, these trajectories did not cluster (see also minimum correlation distances in inset). The observed lack of clustering suggests large differences between the evoked PN response patterns, consistent with our diverse choice of single odors. Adding components to a single odor, W (Fig. 5B), caused the mixture trajectories to deviate from that for W. Incremental changes in the population trajectory, however, decreased as the number of components in the mixture increased (see also minimum correlation distance matrix, inset), consistent with the decrease in the fractional change to the stimulus with each component addition. This observation was repeated with the other odors and quantified by analysis in full PN space (not shown).

While mixture representations deviated from those of their components, they still formed clusters of trajectories, well segregated from those corresponding to non-overlapping mixtures. In Fig. 5C, sets of all single- and mixed-odor trajectories for odors containing only components W, X, Y, and Z and those containing only components A, B, C, and D are plotted, revealing two non-overlapping manifolds, seen as two clusters in inset (A and W).

The qualitative results in Fig. 5A–C suggest that the representations of complex mixtures by PN assemblies are ordered: the more similar odors are, the more similar their corresponding representations. We tested this hypothesis directly by computing metrics in the full (non-dimension-reduced) space. We first computed the dissimilarity between odor stimuli (represented as 8D binary vectors whose coordinates indicate the presence or absence of each of the 8 components) using the Jaccard distance (Deza and Deza, 2009). We computed all pairwise distances $D_j$ between odors, and all correlation distances $D_c$ between the PN population vectors corresponding to those odors (globally, i.e. formed by temporally concatenating the population vectors over the relevant time period). In Fig. 5D we plot the Spearman rank correlation between $D_j$ and $D_c$ calculated over the response (blue), over baseline (red), and a control (gray). During the response window there is a strong tendency for trajectory distances to increase with odor distances (Fig. S5A), while during baseline this trend is very weak. In Fig. S5B we show similar results using two other odor distance metrics. We conclude that the evolving odor representations by the PN population contain information about stimulus composition in their global inter-trajectory distances.

When observed bin-by-bin, distances between trajectories can vary: Fig. 5E, for example, shows the evolution of $D_c$ calculated for odors WYZ and DWYZ over time. But information about odor composition is preserved also in the inter-trajectory distances measured instantaneously. Figure 5F shows the time course of the Spearman rank correlation (calculated as in Fig. 5D) measured piecewise in time. Quickly following odor onset, the correlation reaches a high value of ~0.7 (blue), indicating a strong tendency for trajectory distances to increase with increasing odor distance. The correlation remains high and above baseline value for several seconds after odor offset. Figure S5C shows similar results using the other two odor distance metrics, and Fig. S5D shows the raw data from which the correlations are computed at several times relative to odor onset. We conclude that the responses to mixtures of the PN population continue to contain information about mixture composition in their pairwise distances for several seconds following odor offset. PN

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trajectories do not spread randomly in representation space, a result consistent with those obtained with binary mixtures.

**Kenyon Cell Responses to Mixtures**

Because KCs are the direct targets of PNs in the mushroom bodies, because mushroom bodies are a site for associative memory (Heisenberg, 2003; Masse et al., 2009) and because KC output synapses are plastic (Cassenaer and Laurent, 2007, 2012) KCs are a likely repository of olfactory memories. It is therefore important to determine the stimulus features that they extract from PNs. For comparison, we show first the responses of one representative PN to our 44 stimuli (Fig. 6A). As is typical of PNs (Perez-Orive et al., 2002), see also Fig. S6A), this neuron responded to about half of the stimuli with a variety of discharge patterns. KCs, by contrast, responded very rarely to odors (Fig. S6B) but when they did, did so with very high specificity (KCs 1–3, Fig. 6B, C). Surprisingly, KCs that responded to a component also often responded to many—sometimes all—of the mixtures containing it. KC 1 (Fig. 6B), for example, responded to odor D and all mixtures containing D (though with variations in response onset and duration). The same can be seen with KCs 2 and 6 for odor W (Fig. 6C, D). KCs 5 and 6 were recorded simultaneously, and each responded to a different molecule (C, W). When both odors were included in the mixture (e.g., BCWX, ABCW, BCWZX), both KCs responded, with a few exceptions (ABCDWXYZ). We found KCs specific to all 8 single odors. By chance (see Methods) we also found a few KCs specific for binary mixtures but not their components (not shown).

We used receiver-operator-characteristic (ROC) analysis (Fawcett, 2006) to quantify the response specificity of PNs and KCs, measuring a neuron’s ability to separate stimuli into sets “containing i” and “not containing i”, as response threshold is varied (see Methods). On a true-positive (TP) vs. false-positive (FP) plot, selective neurons are identified by ROC curves that tend towards the corners, while unselective ones run along the diagonal (Fig. 6E, upper panels). The area under the curve (AUC) thus measures selectivity (near 1 or 0 for high, near 0.5 for low) (Fig. 6E, lower panels). This analysis indicated that individual KCs are significantly better (p<10^-10, one-sided Wilcoxon rank-sum test) than individual PNs at component segmentation. These results also held when analyzed in consecutive 50 ms time bins (Fig. 6F). For ~1 second following odor onset, the median of the AUC distribution for KCs is larger than that for the PNs (p < 0.01, one-sided Wilcoxon rank-sum test). Hence, in addition to being highly selective and thus rare responders, single KCs can categorize odors (e.g., as containing X) by extracting component information from PN population vectors.

**Decoding PN Trajectories Over Time**

We next examined the sequential organization of the KC population response. Figure 7A shows three W-responding KCs. As described before, each KC responded at a particular time during the stimulus with just a few action potentials on a baseline of 0. These three KCs also responded to mixtures. Their mixture responses, however, were not identical to their component responses (Fig. 7A). They could vary in intensity, duration and in timing, Figure 7B sorts and plots the PSTHs of KCs that responded to W (grayscale, averaged over 7 trials and normalized) and the peaks of those PSTHs (red). As components were added, the number of mixture-responding KCs increased, in part due to new KCs that detected the other components of the mixture (black dots). The number of responding KCs, while always low (0.5–1% of all recorded KCs in any 50 ms bin), thus increased with mixture size (Fig. S7A). This was correlated not with increased total PN activity—which varies little with concentration (Stopfer et al., 2003) or mixture complexity (Fig. S7A), but rather with increased PN synchronization (Fig. S7B, C). KC responses were distributed during and after the stimulus, with a peak within 200–300 ms of stimulus onset on average (Fig. 7C). The response times of these KCs have been superimposed on the corresponding PN trajectories.
for these three stimuli (Fig. 7D–E), demonstrating that KCs decode PN trajectories throughout their entire duration.

If single KCs each decode segments of PN trajectories, then we should be able to use PN responses to reconstruct those of the KCs. To test this possibility, we formed odor response vectors for each PN and KC by computing the trial-average of spike counts binned in consecutive 100 ms bins in the 1 second window following odor onset, and concatenated across the 44 odors tested, yielding a 440-element response vector for each cell. We then used the multi-stage adaptive lasso (Buhlmann, 2011) to linearly regress the response vector of each KC on those of the PNs, while constraining the regression weights to be positive (Jortner et al., 2007). This is shown schematically in Fig. 7F.

Figure 7G shows three of the best reconstructions: in all three, more than half of KC-response variance could be explained using 30 or fewer of the 174 available PNs. Figure 7H summarizes the distribution of reconstruction results over the KC population. The median value of the fraction of variance unexplained (SSE/SST) is 0.52. Twenty six percent of the KC responses could not be regressed at all on the PN responses available. The mean ± S.E.M. of the number of connections used in the remaining reconstructions was 10 ± 0.6, with a maximum value of 30.

We then tested the mutual consistency of the PN and KC datasets by computing the quality of reconstructions if one or the other set had been shuffled (see Methods). If shuffled PN responses were used (with the same number of regressors as in control), the median of the SSE/SST distribution was 0.82, indicating a significant loss in performance, while using the PN responses to reconstruct the shuffled responses of KCs, yielded a median value of SSE/SST of 0.86, again, indicating a loss in performance. In short, our results show that the responses of single KCs can be reconstructed from pooled subsets of PNs, consistent with the cycle-by-cycle decoding of the PN population by KCs.

Finally, we examined whether the PN trajectories could be reconstructed from the fragments decoded by single KCs by looking for a fixed basis set such that the PN population activity in each time bin could be expressed as a linear combination of basis elements using KC responses as coefficients (Fig. 7I, see Methods). Figure 7J represents the PN odor trajectories corresponding to the odor ABC reconstructed directly from the PN data (blue) or indirectly using the KC data (red), illustrating a good qualitative fit. Figure 7K summarizes the fits over all stimuli and reconstructions. For each time bin along each trajectory, we computed the fraction of the response variance unexplained (SSE/SST), and then averaged this value over the trajectory. The distribution of mean values over the 44 odors is plotted in red. The median value was 0.44, as for the example shown in Fig. 7J. Similarly, we computed the mean over time bins of the correlation between the trajectories and their reconstructions (red, right panel of Fig. 6K). The median was 0.76, the same as that for the example in Fig. 7J.

We next measured the extent to which the recorded KC population was informative about the PNs. We generated sets of shuffled KC responses and for each of 100 shuffles, computed reconstructions of PN trajectories as done with the unshuffled responses. Three of the reconstructions for odor ABC are plotted in Fig. 7J (green), illustrating inferior fits, as confirmed in Fig. 7K. Finally, we tested the extent to which the KCs were well suited for reconstructing the trajectories of recorded PNs. We produced 50 fake PN populations by shuffling the odor responses of single PNs, and reconstructed the trajectories as above using the unshuffled KCs. The distribution of the resulting fit metrics are plotted in gray in Fig. 7K. The median of SSE/SST over all shuffles and all odors is 0.83, again significantly higher (p = 0, rank-sum test) than for the unshuffled data.
In summary, we conclude that all fragments of any PN-population trajectory are decoded by at least some KCs, and conversely, that PN trajectories can be reconstructed from the recorded KC population responses. Thus PN trajectories (formed of dense PN vectors) are mapped onto new trajectories (formed of sparse KC vectors) in KC space, so that each odor (whether it is a mixture or not) is represented by a time series of sparse KC activity vectors.

**Odor Identification, Categorization, and Generalization from Population Activity**

Because KCs are in turn read out by downstream decoders (Cassenaer and Laurent, 2007, 2012; Heisenberg, 2003; MacLeod et al., 1998; Masse et al., 2009), we examined the information present in the KC population vectors in appropriately short time bins. Using a linear classifier (regularized-least-squares, see Methods), we compared the decoding of odor identity, category and generalization using instantaneous PN and KC population output.

Decoding accuracy in the identification and categorization tasks was based on trials excluded from the classifier training; for the odor generalization task, all trials with the tested odor were excluded from training. Thus, the measured accuracy was what real downstream neurons might achieve in single trials by computing a weighted sum of spikes in each measurement bin (see Methods). Identification required attributing a particular KC or PN response vector to the correct odor (all-vs.-all, 44 classes, chance = 2.27%). Categorization consisted in discriminating mixtures containing a given component from those that did not contain it (balanced sets, repeated over all 8 components and averaged; chance = 50%; see Methods). Generalization required the categorization of a previously “unknown” odor (repeated over all positive and negative examples for each of 8 components and averaged; chance = 50%; see Methods). The results are plotted in Fig. 8A–C as a function of time around the stimulus. As expected from our large sample of PNs (~20% of the entire population), identity (Fig. 8A, red) and category (Fig. 8B, red) assignment were nearly perfect (peaks of ~100% and ~90%, respectively) with this PN set, while peak generalization performance (Fig. 8C, red) was slightly lower at ~85%, consistent with it being the more difficult task. KCs could also be read out to perform identification, categorization and generalization. Owing to the small size of our KC sample relative to KC population size (~0.4% vs. 20% for our PN dataset), accuracy was expectedly lower than with PNs. Nevertheless, this performance was only about half that obtained with PN vectors, despite a 40-fold difference in sample size. Randomly subsampling the PNs to match the fraction of KCs sampled typically produced worse performance than that of the KCs (Fig. 8, blue traces), though the best PN subsets could sometimes match KC performance (Fig. S8A). Peak performance was obtained at ~300 ms on average after stimulus onset and remained high for ~500 ms beyond odor offset. Peak categorization and generalization performances (averaged over odors) were reached at similar times with KCs and PNs. The correlation between the time courses of PN and KC read-out accuracy was even more striking when performance profiles were considered individually for each odor (Fig. S8B, C). These observations are consistent with the instantaneous, piecewise decoding of PN output by KCs (Perez-Orive et al., 2004). They also indicate that peak accuracy is not reached with uniform dynamics for all stimuli.

**DISCUSSION**

**Single-PN Responses to Mixtures are Hypo-additive**

By linearly regressing single-PN mixture responses on component responses, we found that most mixture responses could be explained by one component response. This enabled us to decompose the PN populations by component “preference”, showing both that each component is represented in the population, and that a full spectrum of dilution sensitivity exists for each component. Such a distribution of response profiles would not only explain
the global structure in the population activity revealed by LLE (e.g., odor-specific concentration manifolds), but would also clearly facilitate olfactory computation.

The fact that few response fits required multiple components could be a result of the inherent bias of our selection procedure against complex models. Indeed, that procedure always selected a single-component model if the addition of other components did not improve the fit by a value greater than the cost of the model’s increased complexity, even if the physiological truth may have been a mixture response. For binary-mixture experiments, this potential bias was unlikely, because most PNs responded strongly to one component or the other, with relatively few responding to both. In the complex-mixture condition, we examined the ~20% of cases in which at least two equally strong component responses were present but only one was ultimately used in the fit (see supplementary material). In only about 1/2 of these cases did we observe a secondary component response strongly correlated with the primary one (cases that may hence have been rejected by the fitting procedure due to redundancy). We also checked many of the fits “manually”, especially those with high response SNR and poorly fit by single components. These inspections almost always confirmed the decision made. The procedure itself also had few parameters and required little tuning. Thus, we believe that model selection did not introduce a significant bias against multi-component responses.

Component-dominance or ‘hypo-additivity’ has been previously observed in rat olfactory receptor neurons (Duchamp-Viret et al., 2003), M/T cells of the rat olfactory bulb (OB) (Davison and Katz, 2007; Giraudet et al., 2002), mitral cells of the zebrafish OB (Tabor et al., 2004), and at the glomerular level in the honeybee (Deisig et al., 2006) and zebrafish (Tabor et al., 2004). Our observations also support reports of increased inhibition at higher mixture complexity (Davison and Katz, 2007; Deisig et al., 2006; Tabor et al., 2004). Our work advances these studies by examining the responses of a large population of cells to odors at a wide range of mixture complexities, and by explicitly testing for and revealing the extent and diversity of odor and concentration tuning in the PN population.

Previous studies in vertebrates have used models to predict mixture responses of olfactory neurons from responses to components. Giraudet and colleagues regressed mixture responses onto the components and inferred component dominance by the clustering of the regression coefficients (Giraudet et al., 2002). Our method advances this approach by (a) testing all possible combinations of the component responses and (b) using model selection to explicitly choose the model that best balances fit quality and model complexity. Khan and colleagues fit the responses of rat M/T cells to binary mixtures by describing the cell’s response as a linear combination of input currents due to the components scaled by concentration, and excitation caused by air alone, passed through a sigmoidal nonlinearity modeling the current-to-spike-rate function of the neuron (Khan et al., 2008). Their model would be difficult to apply to PNs because the modulation of PN responses with concentration is more complex than those of M/T cells and hard to describe with a simple scaling of a canonical response—hence our decision to use concentration-specific PN responses in our fits.

**Odor Representations by PN Populations are Ordered**

With binary mixtures, measures of similarity between high-dimensional activity vectors (Fig. 3G–I)—constructed from a large sample of antennal lobe PNs—confirmed visual inspection of corresponding trajectories projected into a reduced space after dimensionality reduction (Fig. 3A, D–F): (1) the representation of a 1:1 mixture lies approximately in between those of the components of that mixture; (2) trajectories cluster by odor rather than by concentration; (3) trajectories change smoothly as one odor is morphed into another. Similar experiments on binary mixtures were recently carried out by Niessing and Friedrich.
in zebrafish olfactory bulb, based on simultaneous calcium imaging over large numbers of mitral cells (Niessing and Friedrich, 2010). In that work, two dissimilar amino-acid odors (arginine and histidine) were morphed into one another (as done here), and the corresponding representations compared across all mixture ratios. The authors showed that representations shifted abruptly from that for either component to a mixture cluster—a result in apparent disagreement with ours. Leaving aside the difficulties in comparing two model systems that operate in different media, we believe that the two sets of results are not inconsistent. This is because the abrupt transition to a mixture cluster described by Niessing and Friedrich occurs at mixture ratios between 99:1 and 90:10 (or 1:99 and 10:90), ratios just outside those that we tested (90:10 to 10:90 in steps of 10%). The smooth changes we describe concern trajectories that are entirely within Niessing and Friedrich’s central/mixture cluster, in which average inter-trajectory correlation was about 0.6. A recent study examining the responses of single M/T cells in the olfactory bulb of the anesthetized rat also found gradual rather than abrupt evolution in responses with the mixture ratio (Khan et al., 2008), though again the mixture resolution and range used was coarser than that of Niessing and Friedrich.

With more complex mixtures, we found a positive correlation between chemical similarity (i.e., the number of shared mixture components) and representation similarity. This was true whether representations were measured over the entire response window (Fig. 5D) or piecewise, over individual time bins (Fig. 5F). While such a relationship might have been expected very early in the response, when PN activity is dominated by receptor input, we observed that it obtains throughout the odor presentation and for several seconds following odor offset. Together these results indicate that odor representations are not randomly distributed in PN space, but are ordered so that chemical similarities are reflected in similarities in the evoked neuronal activity patterns. While random representations can in principle be as useful as ordered ones for the decoding of odor identity by downstream cells, ordered representations can make the computation of categorization and generalization easier by representing similar odors in similar ways, and may be the substrate for the categorization and generalization performance we observe in KCs, the PNs’ targets in mushroom bodies.

**Subspace Readout of PNs by KCs**

We used the multi-stage adaptive lasso (Buhlmann, 2011) to regress the binned odor responses of KCs on those of the PNs, and found that by using up to 30 of the available 174 PNs, we could explain ~50% of the variance in KC responses on average. This effective connectivity of ~17% seems to contradict previous electrophysiological results that indicate 50 ± 15% connectivity from PNs to KCs (Jortner et al., 2007). These two results can be reconciled: the sampled PN population was split approximately eight ways according to component sensitivity. Assuming some redundancy between the responses of PNs within a group, a smaller number of ‘basis-PNs’ would be required to capture the variability of all the responses within the group, and only those PNs would show up in the regression (due to the sparseness prior of the lasso). Hence, a low apparent connectivity is probably explained by the redundancy of PN responses.

**Individual KCs are Better Odor Segmenters than Individual PNs**

Surprisingly, Kenyon cells—the postsynaptic targets of PNs—are individually much better than PNs at detecting a component in a mixture of up to eight odors (Fig. 6E, F). Component selectivity was recently reported in the mouse piriform cortex, though mixture complexity was limited to binary mixtures (Stettler and Axel, 2009). KC component selectivity might be explained using a simple abstraction: odor representations are spread orderly in a high-dimensional PN space (Fig. 3,5); because of their 50 ± 15% connectivity to PNs (Jortner et
al., 2007), the KCs each sample a different, lower-dimensional, subspace of PN space. Appropriate choice of these subspaces could allow individual KCs to recognize relationships between the trajectories of odors that contain a common component that are not detectable in the full PN space. Alternatively, given that experiments involving KC recordings only commenced if a response had first been elicited in one KC by at least one of the eight odor components (see below), it is also possible that a yet-to-be-established learning rule fine-tuned PN-KC connectivity during this testing phase, such that a KC was more likely to respond to a mixture if it had recently been exposed to a component of that mixture.

Population Decoding from PNs and KCs

Our results (Fig. 8A–C, S8) show that both the PN and KC populations can be read out by linear classifiers in single time bins to perform odor identification, segmentation, and generalization, and that the time course of the performance is similar in both populations. Although we sampled ~20% of the full PN population, but only ~0.4% of the KC population, readout performance from KCs was usually only slightly worse than from PNs. One explanation for this observation is that by pooling information across the PN population, individual KCs are more informative than individual PNs. However, another explanation is that our bias in KC selection due to experimental design may have skewed our KC dataset towards particularly informative cells (see below). The collection of larger and unbiased KC datasets might elucidate this point.

Experimental Sampling Bias

Because odor representations by PNs are dense, finding responding PNs in an experiment was always guaranteed: recordings could start immediately after tetrodes had been inserted into the antennal lobes and yielded high SNR data. By contrast, the sparse responses of KCs forced us to do a targeted search on KC signal prior to initializing an experiment. This search was done using only the eight single odors in our stimulus set. As soon as high SNR signal was detected on at least one tetrode in response to at least one of the eight odors, the experiment could commence. Our KC dataset is thus somewhat biased towards KCs that respond to the odors present in our single-component stimulus set. One consequence of this bias is that the true response-probabilities of KCs are even lower than we presently estimate. Based on the fraction of recordings in which KCs responding to our 8 single odors were found, the fractions of responding cells we measured in each 50 ms bin (0.5–1%; 5% maximum of our recorded set for one large mixture when integrated over the entire response, Fig. S7A) was likely overestimated 10–20 fold. For example, the 5% maximum response measured for an 8-mixture would in reality correspond to a total of 125–250 KCs (of 50,000) per MB.

Despite our bias towards KCs that responded to the single-component stimulus set, a typical recording session commonly revealed KCs that did not respond to any of the single components. Among those were some that responded to “low-m” mixtures—two or three components. Those KCs then often responded to high-m mixtures containing the low-m ones, practically segmenting these lower-order mixtures, just as other KCs detect single components. Hence, while our KC data are biased towards KCs that responded to the 8 single components in our stimulus set, it contains no experimental bias towards mixture-responsive or component-segmenting KCs, for those were all discovered post-hoc during data analysis, and our database with such KCs is too small to propose statistical estimates of their frequency. It is possible, however, that our initial screening procedure with the eight single odors introduced an acquired (though unconditioned) selectivity for these components. Whether the segmenting properties of KCs we describe here are intrinsic or learned via a fast non-associative process—see for example (Stopfer and Laurent, 1999)—is unknown thus far.
Functional Consequences of Odor Segmentation

That KCs segment components in mixture is important for our understanding of computation in this system. Our results are illustrated in Fig. 8D. Each row represents a KC (taken from our dataset) that expressed good segmenting properties (one KC for each one of the eight single odors). Each column represents one of the 43 stimulus conditions (paraffin oil not shown); color identifies the odor component; circle areas indicate response magnitude. Any odor can thus be represented by a unique 8-D activity vector. To the limit, if every KC was a perfect detector for only one feature, n KCs could encode 2^n-1 different odor feature combinations, plus baseline (0,0,…0). By contrast, a “grandmother” scheme whereby each odor is represented by a unique neuron would require 2^n-1 KCs to represent this many odors and mixtures. Hence, KCs implement a clever strategy. Odor representation is sparse (effective for memory formation and recall, yet not maximally sparse), but distributed such that the coding capacity for related stimuli (mixtures) is greatly increased. This mixture-encoding also simplifies odor generalization by requiring a smaller number of classifiers to compute it (Fig. 8E, F) compared to a more disordered scheme (Fig. 8G–I). One could argue that the coding strategy of PNs is superior, because it engages far fewer neurons (800 vs. 50,000) to accomplish the same goal (information captured by KCs is obviously present across the PN population). However, because PN codes are dense, they overlap extensively. This is economical for encoding, but bad for storage (Field, 1994; Foldiak, 2003) because (among others) different memories would nearly always share synapses and interfere with one another.

We conclude, therefore, that stimuli are not represented by sparse and random sets of KCs. Each KC represents a meaningful feature, and each stimulus is encoded by the combination of relevant feature-selective KCs (Barlow, 1972; Foldiak, 2003). In principle, this ordered scheme allows decoders of KC activity to determine not only the degree of similarity between stimuli, but also the assignment of category for novel stimuli (generalization). Hence, the scheme we observed for mixture coding by KCs is consistent with the fulfillment of several concurrent requirements: economy of size, maximization of capacity for that size, minimization of overlap between memories, and generalization. In summary, we propose that the computational function of the PN-to-KC transformation is a reformatting of odor representations from a dense representation into one that is not only sparse (and hence better suited for learning and memory), but also computationally efficient, in that individual KC responses represent the presence of single odor components, facilitating odor identification, categorization, and generalization. The rules observed here for a simple olfactory system could, in principle, form the basis for the encoding of multi-dimensional signals in any sensory system with comparable requirements.

EXPERIMENTAL PROCEDURES
Experimental Methods

Results were obtained from 61 locusts (Schistocerca americana) and recordings from 342 projection neurons (PNs) and 209 Kenyon cells (KCs) from both antennal lobes (ALs) and mushroom bodies (MBs) in each animal. The brain was prepared as in (Laurent and Davidowitz, 1994). Odors were delivered by injection of a controlled volume of odorized air within a constant stream of desiccated air, using one of two dedicated odor delivery systems (Fig. S1A, B). The odors used were: 1-octanol (A), diluted 0.7 ml/10 ml; phenetole (B), diluted 0.15 ml/15 ml; citral (C), pure 10 ml; benzaldehyde (D), diluted 0.02 ml/15 ml; isoamyl acetate (W), diluted 0.1 ml/10 ml; 2,3-butanedione (X), 0.04 ml/15 ml; 2-nonanone (Y) diluted 2 ml/15 ml; L-carvone (Z), pure 10 ml; paraffin oil (P), pure 15 ml. Extracellular electrophysiological recordings were obtained using wire tetrodes and silicon probes (NeuroNexus) and waveforms were analyzed as in (Pouzat et al., 2002).
Unlike PNs, KCs respond very sparsely to odors and their individual baseline activity is ~1 spike every 30 s on average (Perez-Orive et al., 2002). Hence, significant effort was made to find KCs that responded to some at least of the odors in our panel prior to initializing an experiment. Due to the large number of conditions in our experiments, we did not (nor did we wish to) pre-test all 44 stimuli. Rather, we searched for responsive KCs by presenting the eight monomolecular odors; we selected a recording position from which some spikes could be recorded in response to any one of these stimuli. Due to the rarity of KC spikes, KC-spike cluster models were defined using all trials (usually ~50 conditions, 7 trials each, 14 s per trial). The condition in the middle of the set was used to calculate the noise covariance matrix (Pouzat et al., 2002). The threshold was set typically at 4–5 times each channel’s signal SD. The model generated by this method was refined using criteria identical to those used with the PN data. Stability over the course of the experiment was assessed after sorting and was based on a stable baseline firing-rate over the course of the experiment.

**Recording Constraints and Sampling Biases**

Because PNs respond very promiscuously to odors (Perez-Orive et al., 2002), no effort was made to find PNs that responded to our stimuli. As soon as good signals suggestive of separable PN clusters could be seen, recordings started and responsive PNs were always found. Our estimates of PN response-probabilities are therefore likely close to true values. By contrast, KCs respond very rarely to odors and responsive KCs had to be found through an active search. Experiments would commence only if a response was elicited by at least one of the 8 monomolecular odors. See Discussion for more details.

Further details on experimental techniques and all computational methods are given in Supplemental Information and Figures (S1–8).

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**References**


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Figure 1. Stimulus descriptions
Binary mixture experiments: Citral and 1-octanol were used individually and in various mixtures. Odor pulse was 300 ms, repeated for 10 trials, 14 s / trial. Complex mixture experiments: 8 different molecules and a subset of their mixtures were used. Odor pulse was 500 ms, repeated for 7 trials, 14 s/trial. (A, B) Pure odor concentrations used in binary mixture experiments. (C) Mixture ratios used when morphing octanol to citral. (D) Mixture ratios for concentration series experiments. (E) Complex mixtures: Eight odor components were presented individually and in 2-, 3-, 4-, 5-, 8-component mixtures. Paraffin oil was also presented. Three components (1-octanol, citral, isoamyl acetate) were also presented at 4x concentration, comparable in concentration to 4-mixtures. Number of components indicated at right. See also Fig S1.
Figure 2. Single PN binary mixture responses are complex and hypo-additive

(A–D) Spike rasters showing responses of one PN over 10 trials. 300 ms odor pulses (purple patches). (A) Responses to six concentrations (see Fig. 1A, B) of pure citral (green) and octanol (red). (B) Responses to mixture series (Fig. 1C) from pure octanol (top) to pure citral (bottom). (C) Overlay of observed (filled PSTHs) and expected (sum of responses to components; black lines) PN responses to morphed mixtures. Note mismatch. (D) Responses to the 1:1 mixture ratios in Fig. 1D. (E) Example fit procedure. Data are mean firing rate in adjacent 50 ms bins for the response of the PN in panels A–D to cit140:oct120. Right panels show some fits. Best fit (*) used only citral. (F) Mixture x PN matrices indicating response type of each PN to each mixture. Mixtures are from mostly citral (cit140:oct30, bottom) to mostly octanol (cit30:oct140, top). Colors indicate components used in fit (red: only octanol; green: only citral; yellow: both; black: neither). PNs grouped by type of response dominating their mixture responses. See also Fig S2.
Figure 3. PN population representations of binary mixtures are structured
(A) Dimension reduced (LLE) population trajectories in response to pure citral (green), pure octanol (red), and 1:1 mixture (yellow). (B) Correlation distances between average PN activity patterns in trials 3–6 and trials 7–10 evoked by pure citral, pure octanol, and 1:1 mixture in adjacent 50 ms time bins in 3 s following odor onset. Color range clipped from [0–2] to [0–1] for clarity. (C) Data in (B) summarized as the minimum over each of the 9 odor comparisons, colored as in (B). (D) Same as (A), but now with correlation matrix summary inset. (E) Concentration series trajectories and correlation summary matrix. Concentrations as in Fig. 1D. Citral: dark to light green. Octanol: dark to light red. 1:1 mixture: purple to yellow. (F) Trajectories and correlation summary as pure citral (green) is morphed to pure octanol (red). Concentrations as in Fig. 1C. (G) Time course of mean ± S.E.M. over trials of projection angle fraction (see Methods) with respect to citral for adjacent 100 ms bins starting at −0.5 seconds. Blue patch: 300 ms odor window. PAF for 1:1 mixture remains close to 0.5 after onset, indicating trajectory lies almost exactly in between that of citral and octanol, confirming the impression from Fig. 3A, D. (H) Mean ±
S.E.M. over trials of Rand indices comparing clustering of global trajectories (PN population vectors, 100ms bins, concatenated in time) by correlation distance with clustering by concentration (red), or with clustering by odor (blue), in a 1s baseline period (~1.1s to −0.1s relative to odor onset) (left), and in response window (0.1s to 1.1s relative to odor onset) (right). Dashed lines are the chance levels for each time window (see Methods). At baseline, agreement of clustering by distance with both clustering by odor and by concentration is near the chance level, but in response window, agreement with clustering by odor is very high (0.887 ± 0.006), much higher (one-sided t-test, p<0.001) than with clustering by concentration (0.545 ± 0.003).

(I) Mean (traces) ± S.E.M. (shades) over trials of log_{10} of posterior probability of constant (gray), linear (blue), one-step (red), and two-step (orange) models relative to that of linear model, for evolution of the correlation distance between trajectories for non-pure mixtures and pure citral with the log_{10} of mixture concentration ratio, in 100-ms time bins aligned to odor onset (see Methods). Superiority of the linear model after odor onset indicates PN mixture representations spread smoothly rather than discretely. See also Fig S3.
Figure 4. Single PN representations of complex mixtures are hypo-additive
PN responses to multi-component mixtures (different dataset from Fig. 3: 174 other PNs, stimulated with 44 different odor conditions, see Methods).

(A) Example mixture response fit. Data (left): mean firing rate in adjacent 50 ms bins of a PN to mixture ABCD and its components. Right: Some fits. Best fit (*) is magenta.

(B) PNs arranged by component preference

Legend
- Odor Component Indicator
- Response Types
- Mixture response ~ odor A response
- ~ odor B
- ~ odor C
- ~ odor D
- ~ odor E
- ~ odor W

Response Types
- Mixture response ~ constant.
- Mixture response fit requires multiple component responses.
Figure 5. PN population representations of complex mixtures reflect odor similarity
(A–C) LLE trajectories of population responses. (A) Trajectories for the 8 single-odor components reveal no obvious clustering. (B) Starting from single odor W, trajectories increasingly deviate as components are added (W → WX → WXY → WXYZ → AWXYZ). (C) Mixtures form ordered trajectory clusters. All mixtures composed of W, X, Y, or Z are well separated from those composed of A, B, C, or D. (D–F) Quantification of PN population responses to complex mixtures in the full-D space. (D) Mean ± S.E.M. over trials of Spearman rank correlation ($\rho_{sp}$) between Jaccard distance between odors represented as binary vectors, and correlation distance between global (temporally binned and concatenated in relevant time window) activity patterns (blue), baseline (t= −1.1 to −0.1s relative to odor onset, red), and response window (t= 0.1–1.1s) but with odor labels on trajectories shuffled (gray, near zero). During the response window trajectory distances increase with odor distance ($\rho_{sp}$=0.747±0.004), while at baseline they do so to a much lesser extent ($\rho_{sp}$=0.095±0.017), and not at all if odor labels during the stimulus presentation are shuffled ($\rho_{sp}$=−0.026±0.016). (E) Mean ± S.E.M. over trials of correlation distance between activity patterns evoked by WYZ and DWYZ in adjacent 100ms time windows, starting at t=−0.5 sec. Distance is multiphasic in time. (F) Mean ± S.E.M. over trials of Spearman rank correlation between Jaccard distance between odors represented as binary vectors, and corresponding activity patterns in adjacent 100ms time windows starting at t = −0.5 sec (blue), the activity patterns with odor labels shuffled randomly for each time bin (red), and activity patterns with PN identities shuffled for each bin and each odor (but fixed across trials for a given bin and odor, black). Relationship between odor distances and trajectory distances is strong shortly after odor onset and remains above baseline for several seconds past odor offset, but is absent in baseline and for both shufflings of the data. See also Fig S5.
Figure 6. Single KCs segment odor components better than single PNs

(A–D) Spike rasters of representative PNs and KCs (see Methods for 44 stimuli; 7 trials, 500 ms stimulus at shaded area. Odors organized by number of components (columns) and arranged to maximize neighbor similarity). (A) A typical PN. (B) D-segmenting KC; but note weak late response to Y, WZ, YZ. (C) A W- and a Y-segmenting KC. (D) X-, C-, and W- segmenting KCs. KCs 5 and 6 recorded simultaneously; both also responded (at different times) to mixtures containing both C and W (e.g., BCWX). Only 1 s shown, centered on KC response times. (E) Top panel: ROC evaluation of PN and KC component selectivity (see Methods). Red diagonal: chance performance. Blue lines: results for several example PNs (curve for PN 1 highlighted), and for KCs in (B–D) (curve for KC 1 highlighted). Bottom panel: Distribution of AUC values for KC-odor class pairs is shifted
to the right of PN-odor class pairs \((p<10^{-10}, \text{ one-sided Wilcoxon rank sum test})\). Arrows indicate means: 0.74 (KCs; SD=0.17); 0.59 (PNs; SD=0.20). (F) Time course of AUC distribution for PN-odor and KC-odor pairs in panel E. Median (traces) and 99% confidence intervals of median (binomial distribution approximation, bands) of AUC distribution for all PN-odor (red) and KC-odor (green) pairs, computed for adjacent 50 ms bins. Stars: bins where median for KC-odor pairs was significantly greater \((p<0.01, \text{ one-sided Wilcoxon rank-sum test})\) than for PN-odor pairs. Vertical dashes: odor window; black horizontal: chance level (0.5). See also Fig S6.
Figure 7. Distribution of KC response times, and response reconstructions

(A) KC rasters in response to W, WYZ and ALL (ABCDWXYZ). KC responses are reliable across trials, differ in duration, timing. (B) Activity of all KCs that responded to W, WYZ, and ALL. Gray: Trial-average PSTH normalized to [0–1]; dots: PSTH peaks. KCs ordered by time of peak, illustrating sequential spread of activity, especially tight within first 500 ms. Red dots: W-responding KCs; black dots: KCs that did not respond to W alone. Shaded bar: odor, 500 ms. (C) Instantaneous firing rates computed across all trials and KCs for W, WYZ, and ALL. Values increase with mixture size because more KCs respond to a mixture than to individual components. (D–E) Two-trial averages of LLE trajectories (computed separately for D and E) evoked by W, WYZ and ALL. KC PSTH peaks from (B) are superimposed on PN trajectories at corresponding times. (F) Schematic of KC response reconstruction as non-negative linear combinations of PN responses. (G) Best fits. The three best fits (red) to KC data (blue) are shown. Responses are mean firing rate in 10 adjacent 100 ms bins starting at odor onset, concatenated over the 44 odors (x-label indicates # components per odor). Number of PNs used per fit, fraction of unexplained variance (SSE/SST), and correlation coefficient are indicated. (H) Median, 5'th and 95'th percentiles of SSE/SST and correlation coefficient between responses and reconstructions for each KC. Red: KCs reconstructed from unshuffled PNs; green: KCs from shuffled PNs; gray: shuffled KCs from PNs. (I) Schematic representation of PN trajectory reconstruction from KC responses via a fixed basis Z. (J) Example reconstructions. PN population trajectory for odor ABC is shown in PCA-space (computed using data for all odors) in blue. Fit using KCs is red. Three reconstructions using three different shuffles of the KCs are shown in green. Filled circles: first time bin. (K) Median, 5'th and 95'th percentiles of the mean SSE/SST and
correlation coefficient between responses and reconstructions, over each trajectory. Colors are analogous to panel H. Best performance is when unshuffled KCs are used to reconstruct unshuffled PN trajectories. Median of SSE/SST for fits using shuffled KC responses is 0.67, significantly higher than using the unshuffled data (p = 0, Wilcoxon rank-sum test). See also Fig S7.
Figure 8. PNs and KCs can be linearly decoded to perform odor identification, categorization, and generalization; Coding principles

(A–C) Performance of linear classifiers at decoding odor (A) identity (chance = 0.02), and (B) category (chance = 0.5), and (C) generalizing category (chance = 0.5), as functions of time. Odor pulse between dashed lines (500 ms). Mean (traces) ±3 S.E.M. (bands) across all odor conditions. Results are for PNs (red), KCs (green), and random subset of 8 PNs for which the maximum value (over time) of mean performance was closest to median computed for 100 random subsets (blue). For categorization and generalization, mean performance for the 8 components were first computed, and mean and S.E.M. of these were then plotted. See Fig. S8 for breakdown of mean responses for each of the 8 components.
Coding principles. (D) Responses of eight recorded segmenting KCs (one per each component tested) to all odor conditions, measured as spike counts in 1 s window from odor onset summed over all 7 trials, normalized between 0 and 1 per KC. Circle area represents response magnitude; color represents component. Circle in square indicates “true positive” (correct segmentation); empty square indicates “false negative”; circle alone indicates “false positive”. In the absolute, category decoding requires only 1 KC (e.g. response in KC 3 (horizontal arrow) indicates the presence of odor C almost perfectly. Identification is possible using the 8-D KC vector (e.g., discrimination between mixtures ABCD and ABCDX, vertical arrows). (E,F) Linear classification of odors and mixtures using an ordered KC encoding scheme. Schematic of KC coding space where each KC represents an odor component; generalization is simple. (E) Hyperplanes separating mixtures into A vs. not-A, B vs. not-B, and C vs. not-C; (F) Separation into AB vs. not-AB, BC vs. not-BC, and AC vs. not-AC. (G–I) A scrambled coding scheme. (G) Presence of odors A, B, C, AB, AC and BC can each be computed with a single linear classifier, but A is represented by [0 0 0] and no-odor is represented by activation of KC3, contradicting experiments. (H) A hyperplane exists that separates B from not-B, but AB/not-AB, AC/not-AC and BC/not-BC each require multiple hyperplanes for separation. (I) Odors C and not-C are not separable by a single hyperplane (as shown). See also Fig S8.