Supplemental Information

Encoding of Mixtures in a Simple Olfactory System

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Supplemental Information

The complete experimental datasets presented in this paper, together with the Matlab code used to analyze them can be found at [http://brain.mpg.de/research/laurent-department/software-techniques.html](http://brain.mpg.de/research/laurent-department/software-techniques.html)

**FIGURE S1:**

(A–B) An olfactometer was constructed using small diameter (1/32” inner diameter) Teflon tubing and compression fittings to minimize dead space and delay times. Flow rates of independent air streams were independently controlled by mass flow controllers (range 20–200 ml/min). By mixing odorized air streams with defined flow rates, different mixture ratios were achieved. (A) Binary mixture experiments; (B) Multi–component mixture experiments. (C) Comparison of EAGs for single odors at low and high concentrations.

**Figure S1: Olfactometer setup and EAGs, related to Figure 1.**

- (A) Binary mixture odor delivery
- (B) Multi-component mixture odor delivery
- (C) EAGs for single odors at low and high concentrations
- (D) EAGs for individual single odors
of single odor concentration (8 single odors: 1-Octanol, Phenetole, Citral, Benzaldehyde, Isoamyl Acetate, 2,3-Butanedione, 2-Nonanone, L-Carvone; 5 trials each; 100 ml/min) in black, with EAG of odors presented at 4 times the single odor concentration (3 single odors: 1-Octanol, Citral and Isoamyl Acetate; 5 trials each; 400 ml/min) in red. Shaded region indicates 1 standard deviation above and below the mean. As shown here, single odors were calibrated to elicit minimal EAG response, at the lower end of its dynamic range. EAG shown for two different antennae (i and ii). (D) Comparison of EAG of 8 different single odors, as in (B), and paraffin oil. Concentrations were calibrated to evoke as similar an EAG as possible across single odors, so that no one single odor is dominant during odor mixture conditions. Interestingly, paraffin oil evokes no EAG response in isolated antenna, but can evoke PN and KC responses (see Fig. S5C for KC population response). Shaded region indicates 1 SD above and below the mean (5 trials each). EAG shown for two different antennae (i and ii).
**FIGURE S2:**

(A) Mixture x PN matrices indicating response type of each PN to each mixture. Mixture changes from mostly citral (cit140:oct30, bottom) to mostly octanol (cit30:oct140, top; legend at left). Colors of matrix elements indicate presence of component responses in fit. Red: only octanol; green: only citral; yellow: both;
black: neither. PNs sorted by component dominating their mixture responses: citral-type (left), octanol-type (middle), mixture/ambiguous (right). (B) Fit quality. Matrices arranged exactly as in (A), but colored by fit $R^2$ (legend at left). (C) Mixture response SNR. Matrices arranged exactly as in (A,B) but colored by SNR (legend at left; black: 0 dB – mean response energy equals mean baseline energy; dark-red: 3 dB – mean energy of response deviation from baseline mean is twice that at baseline). Comparing this panel to previous two shows high SNR typically yielded good fits and conversely. (D) Distribution of model fits for each type of response. 1–: unit model, fit is component response (or the sum of the component responses for mixture-type responses) plus constant offset; k–: scaled model, fit is scaled version of component response (or sum of the component responses for mixture-type responses); f: free model, fit is an unconstrained linear combination of component responses; –lag: at least one component used was lagged in time. Note predominance of scaled model in all response types. (E) Effect of mixture on scaling factor of fits. Change in scaling coefficient relative to value at strongest dilution as a function of mixture is plotted using data from all citral-type and octanol-type PNs. Data arranged so that the x-axis labels correspond to same dilution for each response type e.g. data plotted at 140:60 correspond to cit140:oct60 for citral data, and cit60:oct140 for octanol data. Error bars are ±1 S.E.M. Stars indicate significant difference from zero (p<0.05, paired t-test). Dashed lines are sinusoidal fits (citral: $R^2 = 0.92$, $p < 10^{-5}$; octanol: $R^2 = 0.76$, $p < 10^{-3}$). (F) Smoothed histogram of SNR angles (see Supplementary Text) for mixture responses of each type. Values near 0 indicate very weak response to pure citral relative to response to pure octanol; vise-versa for values near $\pi/2$. Most octanol-type responses cluster around 0, most citral type responses cluster around $\pi/2$, indicating lack of response to complementary component.
**Figure S3: Additional information on PN-population binary-mixture response metrics, related to Figure 3.**

(A) Time course of mean (traces) ± S.E.M. (bands) over trials of projection magnitude fraction (length of projection of length-normalized mixture vector on plane spanned by simultaneous component vectors) for the 140:140 binary mixture for adjacent 100 ms bins starting at -0.5 seconds. Odor presentation parameters are as in (Fig. 3G). Mixture vector is mainly in the plane of components for ~1.5 s following odor onset. (B) PAF with respect to octanol.
Values are 1 minus values for PAF with respect to citral (Fig. 3G), confirming mixture vector is between component vectors. (C) As in Fig. 3I, but when using fraction of octanol in the mixture as the independent variable (vs. base–10 logarithm of mixture concentration ratio in Fig. 3I). Linear model is still best over the response window, although the two–step model has a larger posterior than when using the log₁₀ ratio of octanol to citral as the independent variable, due in part to the smaller prior range on the independent variable (~1 vs. ~2). (D) Representative samples of raw data and fits used to produce (E,F). Data are for responses in the fourth trial for time bins starting at the times indicated above each plot. Top row uses log₁₀ ratio of octanol to citral as independent variable (as in Fig. 3I); bottom row uses fraction of octanol (as in (C)). The two sets of plots are very similar because independent variables are approximately linearly related over the range of concentrations tested. Best and second best fits are shown in red and gray, respectively. The log₁₀ posterior probabilities of each model (minus a data–dependent constant term common to all the models) are indicated at top left of each plot, in order of constant, linear, one–step, two–step model; value of best model in bold. Data points for pure citral were not used because correlation distance of pure citral to itself is by definition zero and would introduce an artifact. Data points for pure octanol were not used because the ratio of octanol to citral in pure octanol condition is 1/0 i.e. undefined.
FIGURE S4:

A. Best fits
B. Fit quality
C. SNR

D. Model distribution

E. Scaling factor vs. mixture level

F. SNR angle

G. Weight ratio
**Figure S4: Additional information on single–PN complex–mixture metrics, related to Figure 4.**

(A–G) Further information about single–PN responses to complex mixtures. (A) Component–type of best fits describing PN mixture responses (rearrangement of data in Fig. 4B). Each row is one PN, each column is one mixture. Matrix elements colored according to legend in Fig. 4B. (B) Fit quality. Matrices arranged as in (A), but colored according to R² value of each fit; legend at top. (C) Response SNR. Matrices arranged as in (A,B), but colored according to SNR (dB) of mixture response; legend at top. SNR defined as ratio of average energy of deviations from baseline mean during response, to baseline variance. (D) Distribution of model types according to components used. Each stacked bar plot shows relative frequency of unit– and scaled–coefficient models used in corresponding single component fits, and the distribution of free–coefficient models used in fits where more than one component was used. (E) Scaling factor in fits as a function of mixture complexity. Each panel plots mean scaling factor used in the corresponding single component fits as mixture level is increased. Only PNs which had at least one preferred–component response in at least three mixture levels were used. No obvious trend with mixture level is present in any of the panels. (F) Smoothed histograms of SNR angle (see Supplementary Text) computed over all single–component fits (blue), or limited to PNs with a preferred component and for fits using that component (red). Angles near 0 indicate that the single component response for the component used in the fit was dominant; those near pi/2 indicate that a secondary component response was dominant; those near pi/4 indicate that the primary component response was approximately matched in SNR by at least one of the secondary responses. (G) Smoothed histogram of ratio of scaling weight for best single component fit using secondary component, to that when using primary component, for fraction of data points whose SNR angle (red curve in (F)) was near pi/4. Values below 0.2 were likely to be actively suppressed in the mixture response; those above were likely removed in the fit due to redundancy with the primary component response.
FIGURE S5:

Figure S5: Additional information on PN-population mixture-response metrics, related to Figure 5.

(A) Raw data showing relationship between odor Jaccard distance and the global trajectory correlation distance for each of trials 1–7 (rainbow colored from
blue to red) in the response window. (B) Mean ± S.E.M. over trials of Spearman rank correlation ($\rho_{sp}$) between Braun–Blanquet distance (left) and cosine distance (right) between odors represented as binary vectors, and correlation distance between global (binned and temporally concatenated in response window) activity patterns (blue), baseline ($t = -1.1$ to $-0.1$ s relative to odor onset, red), and response window ($t = 0.1$ to $1.1$ s) but with odor labels on trajectories shuffled (gray, near zero). Results are qualitatively similar to those using the Jaccard distance between odors (Fig. 5D). (C) Mean (trace) ± S.E.M. (band) over trials of Spearman rank correlation of Braun–Blanquet distance (left) or cosine distance (right) between odors represented as binary vectors, and corresponding activity patterns in adjacent 100–ms 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). As in Fig. 5F, immediately after odor onset one observes a strong relationship between odor distances and trajectory distances, that persists for several seconds. The blue traces are very similar across the two panels because Spearman correlation is sensitive to rank–order, not to actual numerical values. Gray patch indicates 500–ms odor window. (D) Raw data for the per-bin relationship between odor Jaccard distance and trajectory correlation distance, at times indicated (odor onset is at 0.0 seconds, reaches the antenna at 0.1 seconds, and remains on for 0.5 second). The trials are colored as in (A).
Figure S6: PN and KC responsivity, related to Figure 6.

The responsivity (see Methods) of PNs (A), and KCs (B), to each of the 43 odors (paraffin oil is not included). Each column represents a single cell; rows are odors organized according to mixture level. (C) Distribution of promiscuity values (total number of odors responded to) for PNs (equivalent to summing the cell’s column in (B)). (D) Same as (C), but for the KCs. KCs are much less responsive than PNs: nearly half of the recorded KCs responded to none of the odors presented, vs. only ~3% for PNs.
FIGURE S7:

A  Time course of PN and KC responses at each mixture level

B  Odor-evoked LFP

C  LFP power vs. KC firing rate per mixture level
Figure S7: Time courses of aggregate PN, KC, and LFP responses, related to Figure 7.

(A) Top: Mean firing rate: Mean PN and KC firing rates as a function of number $n$ of odor components in mixture (black traces; result for each odor at given $n$ are gray; horizontal line is maximum firing rate for single component odors). Firing rate was computed by convolving 10 ms binned spikes with a 20 ms width Gaussian filter. Mean firing rate for PNs remains approximately constant with increasing mixture level $n$; KC mean firing rate clearly increases with $n$. Interestingly, PN firing for single components at 4x concentration is higher than 1x, but no significant differences were detected in KC firing rate.

Middle: percentage of silent cells for each time bin: A cell is defined as silent during a 100–ms time bin if it fired no spike in that time bin in all 7 stimulus trials. Horizontal gray lines are maximum (PNs) and minimum (KCs) level reached for single component odors. Notice that percentage of silent PNs increases as a function of $n$, indicating increased inhibition from LNs, as a form of gain control on the output of the PNs. Peak percentage of silent PNs is reached ~200–300 ms later than peak of PN firing. Four–part odor mixtures elicited greater inhibition than single components at comparable concentration, suggesting that the form of gain control observed here is specialized for mixtures. In comparison, at baseline all KCs are silent. The silent fraction dips to ~90% for single components and returns to near complete silence within 500 ms of odor onset, which we attribute to feedback gain mechanism of (among others) a Giant GABAAergic Neuron, keeping KC responses sparse.

Bottom: Percentage of responsive PN– and KC–odor pairs as a function of odor components in consecutive 50–ms time bins: Very few PNs and KCs were responsive at baseline by our measure (see response metric in Methods). However, shortly after odor onset, 7–14% of PNs were responsive, compared to 0.5–1% of KCs in any 50–ms time bin. Numbers in upper right show cumulative proportion of responding cells over 3 s.

(B) Odor–evoked LFP. Larger odor mixtures (as components in mixture increases) elicit greater oscillatory power in the local field potential (LFP). Single odor components (at 1x concentration) elicit minimal (if any) power in LFP (left most
column). Shown single trial (from 1 experiment) to all odor conditions. LFP was band-pass filtered, 5–35 Hz. Odor bar indicates time since odor onset; odors arrive at the antenna about 100 ms later.

(C) LFP power vs. mean KC firing rate at each mixture level. Mean normalized LFP power in 5–35 Hz band (mean over all odors and 6 locusts, 200–ms sliding window, 50–ms steps) in black line (SD in gray). Mean instantaneous KC firing rate (10–ms–binned spikes convolved with 20–ms Gaussian) is superimposed in red. Increases in both KC firing rate and LFP power are well matched. Notice that increases in LFP power with \( n \) components in the mixture cannot be explained by concentration alone, as 4–part odor mixtures (4–) elicit still greater power than single odors at 4x the concentration (4x 1–) (the two are at equivalent concentration and elicit equivalent EAG responses, not shown). Interestingly, Paraffin oil does not elicit power in the LFP or EAG responses (see Fig. S1), but does lead to increases in KC firing rate. Odor bar shifted by 100 ms to better align with odor arrival at the antenna.
FIGURE S8:

A  Readout performance using random PN subsets

B  Time course of categorization performance per odor component

C  Time course of generalization performance per odor component
**Figure S8: Performance of random PN subsets, and time course of categorization and generalization performance for PNs and KCs, related to Figure 8.**

(A) Performance of random PN subsets on odor identification, categorization, and generalization. Same data as Fig. 7, but showing only mean accuracy traces, and showing results for the two random PN subsets with best (dark blue), worst (light blue), and median (medium blue) peak performance, with performance of a subset computed as the maximum value (over time) of mean accuracy. (B–C) Time course of categorization and generalization performance per component for PNs and KCs. In each time-bin, mean classification performance was computed over all withheld trials (categorization) or odors (generalization), for each of the two valences (in–category and not—in–category) separately. The mean (traces) and S.E.M. (bands) over 50 random sub–samples without replacement of the training trials (to equalize in– and out–group sizes; see Supplementary Methods) and both valences of the mean performance for each component is shown for PNs (red) and KCs (green). (B) Time course of categorization performance. Decoding accuracy of KCs follows faithfully that of PNs, except for odor component ‘C’, where peak accuracy KCs occurs ~500 ms before that of PNs. Timing of peak accuracy varies across odor component categories, occurring within 100 ms of odor onset for ‘W’, but as late as ~200 ms after odor offset for ‘A’. Thus KCs as a population could extract different stimulus features at different favored times. Dots indicate time of peak performance. Vertical blue lines indicate odor onset and offset (500 ms pulse). Chance performance at 50%. (C) Time course of generalization performance. Time course of decoding accuracy is very similar to that for categorization, but with lower performance due to the increased difficulty of the generalization task relative to categorization. Dots indicate time of peak performance. Vertical blue lines indicate odor onset and offset (500–ms pulse). Chance performance at 50%.
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Preparation and Stimuli
Results were obtained from 61 locusts (*Schistocerca americana*) in a crowded colony. We recorded from 168 PNs for the binary mixture experiments, 174 PNs for the multi-component mixture experiments and 209 KCs from 13 (42 groups), 11 (36 groups) and 37 locusts (53 groups) respectively. Experiments were typically conducted using left and right antennal lobes (ALs) and mushroom bodies (MBs) in each animal. Young adults of either sex were immobilized, with one or two antennae intact for olfactory stimulation. The brain was exposed, desheathed and superfused with locust saline, as previously described (Laurent and Davidowitz, 1994). Odors were delivered by injection of a controlled volume of odorized air within a constant stream of desiccated air. Teflon tubing was used at and downstream from the mixing point (see below) to prevent odor lingering and cross-contamination. Due to different odor stimulus requirements between the binary morphing and the multi-component mixture experiments, we built two independent odor delivery systems (Fig. S1A,B).

Binary–Mixture Experiments
Two odorants (1-octanol and citral, Sigma, Fig. 1A–D) were stored as pure solutions in independent 500 ml custom-made bubblers. The odor nozzle (1 cm diameter, Teflon) was placed 1 cm from the antenna and supplied a constant 1 l/min carrier stream of desiccated, filtered air. The flow of each odor was controlled by an independent electronic flow meter with feedback control (Aalborg, 2–200 ml/min) and a solenoid placed upstream of custom-made bubbler (Fig. S1A). Relative odor concentrations were varied by controlling flow through each bubbler (30, 60, 80, 100, 120 and 140 ml/min). These concentrations spanned the dynamic range of electro-antennal responses recorded in recordings from isolated antennae (electro-antennograms, or EAGs). A large vacuum hose placed behind the antenna guaranteed the quick removal of odorants from the space surrounding the antenna. Odor puffs were triggered automatically using a custom computer interface (LabView, National Instruments Inc.). Trials were 14 s long, with 300–ms–long odor puffs presented 2 s after trial onset; each odor condition was repeated 10 times.
**Complex Mixture Experiments**

Individual odors (Fig. 1E) were chosen to be chemically different and their respective concentrations adjusted to ensure that no one odor dominated over the others due to intrinsic differences in vapor pressure. In practice, odor concentrations were calibrated by dilution in paraffin oil to equalize EAG responses, recorded from isolated antennae (Fig. S1D). Paraffin oil alone elicited negligible EAG response. The odors 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; iso-amyl-acetate (W), diluted 0.1 ml/10 ml; 2,3-butanedione (X), 0.04 ml/15 ml; 2-nonenone (Y) diluted 2 ml/15 ml; L-carvone (Z), pure 10 ml; paraffin oil (P), pure 15 ml. The individual odors were each placed into a glass vial (60 ml). The headspace content was carried by puffs of desiccated and filtered air, with a flow rate of 100 ml/min for individual odors and 400 ml/min for paraffin oil. Three odors: 1-octanol (A), citral (C), and iso-amyl-acetate (W) were also presented at a second, higher concentration, by increasing flow rate to 400 ml/min. Odor mixtures were presented by combining the single odorants. For example, AB was the combination of 100 ml/min of A with 100 ml/min of B, with a total odor flow of 200 ml/min; thus, total odor concentration was higher during mixture conditions. A compensating stream of desiccated air was used to ensure that total airflow remained constant throughout the experiment. The odors were mixed in a custom-built corrugated glass tube (~8 cm long, 1.5 cm diameter), with a total flow of 2 l/min to ensure turbulent mixing. The individual odor lines were arranged along the circumference of the mixer. Trials were 14 s long, with odor puffs presented for 500 ms, 2 s after trial onset, and repeated 7 times with each stimulus. To minimize the potential effects of priming (Bäcker, 2002), single odorants were presented first, followed by 2-, 3-, 4-, 5- and 8- mixtures. The order of presentation within each mixture group was pseudo-random.

**Electrophysiology**

Two types of tetrodes were used for extracellular recordings. Silicon probes were obtained from NeuroNexus. Wire tetrodes were constructed with insulated 0.0005" and 0.0004" wire (REDIOHM wire with PAC insulation). Four strands of
wire were twisted together and heated to partially melt the insulation. The tip was cut with fine scissors and each channel tip was electroplated with a gold solution to reduce the impedance to between 200 and 250 kΩ at 1 kHz. The same custom-built 16-channel preamplifier and amplifier were used for both types of tetrodes. Two to four tetrodes were used simultaneously. The preamplifier had a gain of 1, and the amplifier gain was set to 10,000. Because of low baseline activity and low response probability in KCs, fewer KCs than PNs were usually isolated in a typical recording session. Tetrodes were placed within the AL or MB soma clusters, peripheral to the neuropils at depths between 50 and 200 μm. For some MB recordings (KCs, LFP), probes were pressed on the surface of the MB. Cell identification was unambiguous because PNs are the only spiking neurons in the locust AL—LNs do not produce sodium action potentials (Laurent and Davidowitz, 1994)—and because all the somata located dorsal to the MB calyx belong to KCs. Recording locations were tested randomly across the MB and selected if activity could be elicited by any of the 44 odor conditions. Identical stimuli were presented at the beginning, middle and end of the experiment to check that clusters had not drifted significantly over the course of the experiment. Drift was estimated qualitatively by determining if a given neuron’s responses to each odor were similar across these three sampling periods. Hints of drift then led to examination of the waveform clusters.

**Extracellular Data Analysis**

Tetrode recordings were analyzed as described in (Pouzat et al., 2002). Briefly, data from each tetrode were acquired continuously from the four channels (15 kHz/channel, 12 bit/sample), filtered (custom-built amplifiers, band-pass 0.3–6 kHz) and stored. Events were detected on all channels as voltage peaks above a pre-set threshold (usually 2.5–3.5 times each channel’s signal SD for PNs and 4–5 SDs for KCs). For any detected event on any channel, the same 3 ms window (each containing 45 samples) centered on that peak was extracted from each one of the four channels in a tetrode. Each event was then represented as a 180-dimensional vector (4 × 45 samples). Noise properties for the recording were estimated from all the recording segments between detected events, by computing the auto- and cross-correlations of all four channels. A noise covariance matrix was computed and used for noise whitening. Events were then
clustered using a modification of the Expectation–Maximization algorithm. Because of noise whitening, clusters consisting of, and only of, all the spikes from a single source should form a Gaussian (SD = 1) distribution in 180-dimensional space. This property enabled us to perform several statistical tests to select only units that met rigorous quantitative criteria of isolation (Pouzat et al., 2002).

SUPPLEMENTAL COMPUTATIONAL PROCEDURES

MATLAB (The MathWorks, Inc.) was used for all data analyses.

Integration for Bayesian Model Selection

We used Bayesian model selection (MacKay, 2003) to select among models describing our data. Given two models $M_1$ and $M_2$ of data $D$, posterior probabilities $P(M_1|D)$ and $P(M_2|D)$ are computed and model $M_1$ selected as a better description of the data than $M_2$ if the ratio $P(M_1|D)/P(M_2|D)$ is greater than 1. Applying Bayes’ Rule, this ratio is

\[
\frac{P(M_1|D)}{P(M_2|D)} = \frac{P(M_1)P(D|M_1)/P(D)}{P(M_2)P(D|M_2)/P(D)} = \frac{P(M_1)\int_{\theta_1} P(D, \theta_1|M_1) \, d\theta_1}{P(M_2)\int_{\theta_2} P(D, \theta_2|M_2) \, d\theta_2}
\]

where $\theta_1$ and $\theta_2$ are place-holders for the parameters of the two models. The parameter likelihoods $P(D|\theta, M)$, parameter priors $P(\theta|M)$, and model priors $P(M)$ depend on the form of each model $M$.

The required posterior probabilities are computed via the evaluation of integrals over model parameters. Unless otherwise noted, the integrals were computed using Laplace’s method (MacKay, 2003), in which a Gaussian is fit to the integrand at its peak and the integral is estimated as that of the fit Gaussian. The peak of the integrand was found numerically using the \texttt{fminsearch} function in MATLAB’s Optimization Toolbox, and the Hessian of the logarithm of the integrand (required for the Gaussian fit) was estimated using the \texttt{hessian} function.
provided in John D’Errico’s DERIVEST toolbox, available on the MATLAB file exchange.

**Explaining Single PN Mixture Responses**

The procedure for fitting single PN binary mixture morph responses was the same as when fitting complex mixture responses. We first binned the responses into consecutive 50 ms time bins starting 0.5 sec before odor onset and ending 3 seconds after odor onset, for a total of 70 bins. We chose a window of this size so that the models fit would have to explain the transitions to and from baseline as well as the response itself. The spike counts in these bins were averaged across trials, yielding, for an \( n \)-component mixture, \( n \) 70-element regressors (the responses to the single components), and 1 70-element response to be fit. We then computed posterior probabilities for the constant model (no component responses used) and each of the \( 2^n - 1 \) regressor configurations in which at least one of the component responses was used. For each of these configurations, we tested the following three types of models:

<table>
<thead>
<tr>
<th>Name</th>
<th>Form</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit</td>
<td>( \text{Fit}(t) = b_0 + x_1(t) + x_2(t) + \ldots )</td>
<td>( b_0 )</td>
</tr>
<tr>
<td>Scaled</td>
<td>( \text{Fit}(t) = b_0 + b_1 (x_1(t) + \ldots + x_k(t)) )</td>
<td>( b_0, b_1 )</td>
</tr>
<tr>
<td>Free</td>
<td>( \text{Fit}(t) = b_0 + b_1 x_1(t) + \ldots + b_k x_k(t) )</td>
<td>( b_0, b_1, b_2, \ldots, b_k )</td>
</tr>
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The models are distinguished by the constraints they place on the regressor coefficients. The Unit model forces the coefficients to be 1. The Scaled model allows a non-unit coefficient for the regressors but requires that the value be the same for all components. The Free model allows each regressor to have its own coefficient. For computing the model posteriors, we used the standard linear regression assumptions that the data points in each time bin are independent and normally distributed around their predicted values with variance \( \nu \) (an additional unknown parameter). We could then compute the likelihood of the parameters \( (b, \nu) \) given the data \( y \) for the model \( M \) as

\[
P(y|b, \nu, M) = \prod_{t=1}^{T} \frac{1}{\sqrt{2\pi\nu}} \exp \left( -\frac{(y(t) - \text{fit}(t))^2}{2\nu} \right) = (2\pi\nu)^{-T/2} \exp \left( -\frac{\text{SSE}}{2\nu} \right)
\]
where SSE is the sum of the squared difference between the fit and the data. We used the same, Gaussian, prior for the regression coefficients, and an inverse Gamma prior for the variance:

\[
P(b, v|M) = P(b|M)P(v|M)
\]

\[
P(b|M; \sigma_b^2) = \prod_{i=1}^{k} \frac{1}{\sqrt{2\pi\sigma_b^2}} \exp\left(-\frac{b_i^2}{2\sigma_b^2}\right) = (2\pi\sigma_b^2)^{-k/2} \exp\left(-\sum_{i=1}^{k} \frac{b_i^2}{2\sigma_b^2}\right)
\]

\[
P(v|M; \alpha, \beta) = \beta^n v^{-\alpha-1} e^{-1/\nu} \frac{1}{\Gamma(\alpha)}
\]

We set the variance of the regression coefficients prior after earlier exploration with unconstrained fits showed that the coefficients typically fell in the \([-1.5, 1.5]\) range: a variance of 2 would allow the regressions freedom to achieve these coefficients. For the inverse Gamma prior on the noise variance, we set alpha and beta to 1. We then computed the likelihood of each model by using Laplace’s method to approximate the integration over model parameters:

\[
P(y|M) = \int_0^\theta P(y, \theta|M) d\theta = \int_0^\theta P(y|\theta, M)P(\theta|M)d\theta
\]

We were then in a position to compute the posterior on each model given the data, to within a constant of proportionality (the same for all models):

\[
P(M|y) = \frac{P(y|M)P(M)}{P(y)} \propto P(y|M)P(M)
\]

In our first attempt we used the same prior for all models, so that the model with the highest likelihood was selected. However, manual inspection of the fits showed that non-constant models were being chosen where the constant model would suffice, and spurious secondary components were frequently included in the fits. After an initial period of exploration with manual tuning of the model priors, we took inspiration from the minimum description length principle (Grünwald, 2007), and assigned a model fitting an \(n\)-component mixture with \(k\) components a prior proportional to \(n^{-k}\) since, in a naive encoding, \(\log(n)\) bits would be required to specify each of the components used \((k \log(n)\) bits in total). The log probability of the prior on a model would then reflect its description cost, so that \(\log(P(M)) = c - k \log(n)\), yielding the expression above for the prior probability on the model. Using this prior on models immediately produced very good results and obviated manual tuning of the model priors.
Finally, to fit lagged versions of the models, we first estimated the best lag values by trying all possible lag combinations for the single components being considered in a given fit (shifting each component by up to 3 time bins in either direction), and taking the combination that produced the best ordinary least-squares fit to the data. We then substituted these lagged versions of the regressors in the fitting procedure above, while also including terms for the priors on lags. We used a Gaussian prior with mean zero and variance 1 for each lag term, to represent our belief that small lag values may be possible due to jitter in responses and odor delivery, but that large lag values are likely to be spurious.

Using the procedure described above we could compute posteriors for all of the models given the observed data (to within a same constant of proportionality for all models), and we chose the model with the highest posterior.

**Dimensionality Reduction**

For nonlinear dimensionality reduction of PN population activity with Locally Linear Embedding (Roweis and Saul, 2000), we used code from Sam Roweis (http://www.cs.toronto.edu/~roweis/lle/) with Gerard Sleijpen's code for the JDQR eigensolver (http://www.math.uu.nl/people/vorst/JDQR.html). We used as inputs 168–D (1–D per PN), 50 ms time slices averaged over 3 trials (binary mixtures), and 175–D (one PN in this dataset was found to be a spike-sorting duplicate; qualitative effect on LLE plots should be minimal as effect on required distance metrics is slight; all other PN metrics in manuscript, including correlation–distance insets and other full–space verifications of LLE patterns (see Fig. 3G–I, 5D–F) were performed without the duplicate PN), 50 ms time slices averaged over 2 trials (complex mixtures). Other details are as described in (Stopfer et al., 2003).

**Single PN Response SNR**

To compute the SNR of a single PN’s mixture response, we first computed the average noise power by computing the variance of each component response and the mixture response during the baseline period. The baseline period was defined as the two–seconds before odor onset, and the 5–second interval starting 8
seconds after odor onset (this latter interval was selected because the average activity computed over the population had settled to near its baseline value by this time). The baseline variance was then averaged over the components and the mixture response to yield an estimate of the noise power. The signal power for a given response was defined as the average of its squared deviation from its mean value during the baseline period, over the two seconds following odor onset, \textit{i.e.}, the mean squared-error when using the baseline mean to predict the response. The signal power was then divided by the noise power to yield the SNR. To get the SNR in dB, its base-10 logarithm was multiplied by 10. SNRs for component responses were computed similarly, but substituting the component response for the mixture response when computing the signal power.

\textbf{Correlation Distance Insets}

To complement the LLE trajectories in low-D space for PN odor responses, we computed correlation distances (1 minus linear correlation) between mean odor responses (# PN-dimensional rate vectors for each time bin) in trials 3–6 vs. trials 7–10 for the binary mixtures, and trials 2–4 vs. 5–7 for the complex mixtures, in consecutive 50 ms time bins starting at odor onset. We chose to use correlation distance instead of correlation because it is a measure of distance, which is more natural when discussing relationships between trajectories. Euclidean distance is not suitable because (a) it is sensitive to the mean firing rate of the population, while we are interested in deviations from the mean, and (b) a population–mean–subtracted Euclidean distance would still be sensitive to the magnitude of deviations from the mean. Previous work and the present one indicate that it is the direction of the deviations that signals the identity of odors. Since most of our work was concerned with the encoding of odor identity, we felt that correlation distance was the more appropriate measure to use, as it is only sensitive to the directions of deviations.

Figure 3B shows the resulting distances for the responses to pure octanol, pure citral, and their 1:1 mixture. To summarize this information, we then computed the minimum value of the distance for each odor comparison (Fig. 3C), and included this summary inset with each one of our LLE figures. The displayed range of the distances was clipped for clarity to 0–1 from the full 0–2.
**PN Population Odor Representation Metrics**

**Conventions**
Unless otherwise noted, we used the following conventions for the PN odor representation metrics. The *response window* refers to the 1-second period [0.1 s to 1.1 s] following odor onset. The 100 ms offset is to account for the time needed by odors to reach the antenna. The *baseline window* refers to the 1-second period [-1.1 s to -0.1 s] before odor onset. Responses were binned in 100 ms consecutive bins aligned with odor onset. Metrics were computed for single trials, and means and S.E.M.s were computed across trials. Metrics were computed either *locally*, meaning independently for each time bin, or *globally*, by first temporally concatenating the binned responses in the window into a single vector (the *global trajectory*), and then computing the metrics.

**Correlation Distance Insets**
Correlation distances were computed between mean odor responses in trials 3–6 vs. 7–10 in consecutive 50 ms bins, starting at odor onset, and summarized using the minimum value in each odor comparison. The data range was clipped for clarity from [0–2] to [0–1].

**Projection Angle Fraction**
To compute the Projection Angle Fraction (PAF) of a binary mixture response with respect to citral, the mixture response in a given bin was projected onto the plane spanned by the simultaneous responses to pure octanol and to pure citral. The (positive) angle of the projection to citral was computed, and divided by the (positive) angle between the vectors for octanol and citral. The PAF with respect to octanol was computed similarly, except that the angle of the projection to octanol was used instead of that to citral.

**Concentration Series Clustering**
We used the Rand index (Rand, 1971) to determine whether the population responses to the binary mixture concentration series clustered by odor or by concentration. Responses for each odor were binned in 100–ms bins and temporally concatenated in the 1s following (or preceding) odor onset. Given two
partitions (non-overlapping and exhaustive clusterings) $L_1$ and $L_2$ of a finite dataset, the Rand index is defined as the ratio of the sum of the number of pairs of elements that are in the same partition in both datasets and the number of pairs that are in different partitions in both datasets, to the total number of pairs of elements. It ranges in value from 0 to 1, with higher values indicating greater agreement between the two partitions. Intuitively, it can be interpreted as the probability that a randomly selected pair of elements will be grouped the same way in both clusterings.

In line with the dimensionality reduction results (Fig. 3E), we assumed that the global trajectories in a given trial for the three odors and the five concentrations would form three clusters when clustered by correlation distance. Our aim was to use the Rand index to measure the agreement of such a clustering with clustering by odor (by labeling each trajectory with its corresponding odor), and with clustering by concentration (by labeling each trajectory with its corresponding concentration). Because trajectories for 5 concentrations were available, such a procedure would introduce a bias against clustering by concentration due to the mismatch in the number of clusters (3 for clustering by distance, 5 for clustering by concentration). Hence we first split the data into all 10 possible subsets of 3 of the 5 concentrations (no such procedure was necessary for the clustering by odor, since only three odors were used). For each concentration subset, we used 10 runs of k-means clustering to cluster the nine global trajectories ($9 = 3$ odors $\times 3$ concentrations) in a time window of interest for a single trial into three clusters by correlation distance. For each such run, we computed the Rand index measuring the agreement of this clustering with clustering by odor, and another Rand index measuring the agreement with clustering by concentration. We then computed the averages of these two indices over concentration subsets and k-means runs to yield two summary indices for each trial. Finally, we computed the means and S.E.M.s of these two summary indices over trials to yield the bar plots in Fig. S3D.

Because the Rand index does not correct for chance (e.g. by assigning the chance level of agreement a value of 0), we estimated the chance level directly. (An “adjusted Rand index” exists that performs such a correction (Hubert and Arabie, 1985), at the cost of making the index itself more complex. For clarity of interpretation, we chose to use the simple Rand index and compute the chance level directly.) For each of the concentration subsets and k-means runs above, we
computed a chance Rand index by randomly shuffling the odor (or equivalently, concentration) labels of the trajectories, and computing the index between the resulting clustering and that by correlation distance. We repeated this procedure 1000 times for each run. We then averaged the indices over these repetitions, the 10 concentration subsets and the 10 k-means runs to yield a summary index for the chance level in each trial. We then computed the mean and S.E.M. across trials, and displayed the means for the two different time windows of interest in Fig. S3D (the S.E.M.s were negligible and were left out for clarity). The slight difference in the chance levels between the baseline and response windows is due to the differences in clustering by correlation distance in the two time windows.

PN Population Trajectory Evolution
To characterize the spread of PN odor representations when pure citral was morphed to pure octanol we fit, for each 100ms bin, the correlation distance from the mixture population response vector to that for pure citral as a function of (i) log_{10} concentration of citral to octanol, or (ii) fraction of octanol in the mixture, using four different models (specifying the parameter likelihood functions; see table below): a constant model, a linear model, a one–step model, and a two–step model, and used Bayesian model selection to select between them. All models used assumed that data points are normally distributed independently with variance \( \nu \) around the model–dependent mean function of the independent variable \( x \). The form of each model, along with the prior on its parameters and the integration method used to compute the posterior, are tabulated below:
where the data points are \((x[i], y[i])\) for \(i = 1, 2, \ldots, K\), \(x_l\) and \(x_u\) are the lower and upper limits of the prior range on \(x\) and similarly for \(y_l\) and \(y_u\), \(\Delta x = x_u - x_l\) is the width of the prior range of \(x\) values and similarly for \(\Delta y\), \(\nu\) is the variance, \(m\) and \(b\) are the slope and offset of the linear model, \(y_1, y_2,\) and \(y_3\) are the various constant values of the constant and step models, \(\theta_1\) and \(\theta_2\) are threshold values defining step boundaries, and \(K_1\) and \(K_2\) are the number of elements constituting each step. Priors on the constant levels and the intercept of the linear model were uniform over the \([0, 2]\) range of correlation distance. The prior on the slope of the linear model was taken to yield a uniform prior over \([-\pi/2, \pi/2]\] on the angle of the line (relative to the \(x\)-axis). For the one- and two-step models, the prior on the first threshold value was uniform over the \(x\)-range of the data, and that on the second was uniform over the range from the first threshold to the upper limit of the \(x\)-range. An “improper” prior (Sivia and Skilling, 2006) of \(1/\nu\) was used for the variance. The forms of the models and the assumed independence of the data points yielded likelihoods for each model of \((2\pi\nu)^{-K/2} \exp(-SS_T/2\nu)\), where \(SS_T\) is the sum of the squared deviation of the data points from the mean predicted by the model. For the constant and linear models, the integral in the likelihood equation above was computed using Laplace’ method. The thresholds in the one- and two-step models make their integrands only piecewise continuous, so the integration was performed by first applying Laplace’s method to the pieces and then integrating the result over the pieces either directly in the case of the one-step model, or using Monte Carlo integration (with 10,000 randomly selected points in the integration range) in the case of the two-step model.

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters</th>
<th>Form</th>
<th>Prior$^{-1}$</th>
<th>(SS_T)</th>
<th>Integration Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>(v \in (0, \infty)) (m \in (-\infty, \infty)) (b \in [y_l, y_u])</td>
<td>(y[i] \sim N(mx[i] + b, \nu))</td>
<td>(v(\Delta y)\pi(1 + \nu^2))</td>
<td>(\sum_{i=1}^{K}(y[i] - mx[i] - b)^2)</td>
<td>Laplace’s Method</td>
</tr>
<tr>
<td>Constant</td>
<td>(v \in (0, \infty)) (y_i \in [y_l, y_u])</td>
<td>(y[i] \sim N(y_i, \nu))</td>
<td>(v\Delta y)</td>
<td>(\sum_{i=1}^{K}(y[i] - y_i)^2)</td>
<td>Laplace’s Method</td>
</tr>
<tr>
<td>One-step</td>
<td>(v \in (0, \infty)) (y_i \in [y_l, y_u]) (\theta \in [x_l, x_u])</td>
<td>(y[i] \sim \begin{cases} N(y_1, \nu) &amp; x[i] &lt; \theta \ N(y_2, \nu) &amp; x[i] \geq \theta \end{cases})</td>
<td>(v(\Delta y)^2\Delta x)</td>
<td>(\sum_{i=1}^{K_1}(y[i] - y_1)^2 + \sum_{i=K_1}^{K_2}(y[i] - y_2)^2)</td>
<td>Laplace’s Method (over (y_1, y_2) and (v) (jointly) to yield (Q(\theta)), followed by direct integration of (Q(\theta)) over (\theta).)</td>
</tr>
<tr>
<td>Two-step</td>
<td>(v \in (0, \infty)) (y_1 \in [y_l, y_u]) (y_2 \in [y_l, y_u]) (y_3 \in [y_l, y_u]) (\theta_1 \in [x_l, x_u]) (\theta_2 \in (\theta_1, x_u])</td>
<td>(y[i] \sim \begin{cases} N(y_1, \nu) &amp; x[i] &lt; \theta_1 \ N(y_2, \nu) &amp; \theta_1 \leq x[i] &lt; \theta_2 \ N(y_3, \nu) &amp; x[i] \geq \theta_2 \end{cases})</td>
<td>(v(\Delta y)^2\Delta x(x_u - \theta_1))</td>
<td>(\sum_{i=1}^{K_1}(y[i] - y_1)^2 + \sum_{i=K_1}^{K_2}(y[i] - y_2)^2 + \sum_{i=K_2}^{K_3}(y[i] - y_3)^2)</td>
<td>Laplace’s Method (over (y_1, y_2, y_3) and (v) (jointly) to yield (Q(\theta_1, \theta_2)), followed by Monte Carlo integration of (Q(\theta_1, \theta_2)) over (\theta_1) and (\theta_2).)</td>
</tr>
</tbody>
</table>

\(28\)
**Odor Metrics**

There is no obvious metric for measuring similarity between odors. Hence for our complex mixtures, which we represented as 8–bit binary vectors whose elements indicate the presence of each of the 8 single odor components, we tried three different metrics that satisfy two intuitive criteria: (1) odors that do not share any components should be maximally dissimilar, and (2) for a fixed amount of overlap between two odors, the distance should increase as the number of components in the odors increases. The metric we used in the main text is the Jaccard distance (Deza and Deza, 2009), defined for two binary vectors $x$ and $y$ as:

$$
{d}_J(x,y) = 1 - \frac{|x \land y|}{|x \lor y|},
$$

where $\land$ is bit–wise logical AND, $\lor$ is bit–wise logical OR, and $|x|$ is the $L_1$ norm (the number of 1’s in the binary vector $x$). When comparing two odor vectors, this distance is 1 minus the ratio of the number of odor components the two odors share to the total number of components present pooled over the two odors. This metric satisfies both of our criteria above, since (a) if two odors don’t share any components, then the numerator of the ratio will be zero, and the distance assigned will be 1 (maximal), and (b) if the numerator is held constant while the number of components in either odor is increased, the denominator in the ratio will increase, reducing the ratio and increasing the distance.

The cosine distance also satisfies our criteria:

$$
{d}_C(x,y) = 1 - \frac{x \cdot y}{|x||y|}.
$$

Here the norms $|x|$ are Euclidean norms. If the odors do not share any component, their dot product is zero and the distance is maximal; if the dot product is kept constant but the number of components in either odor mixture is increased, then its norm will increase, the ratio will decrease, and the distance will increase.

Finally, we tried the Braun–Blanquet (Deza and Deza, 2009) distance, defined as
$$d_{BB}(x,y) = 1 - \frac{x \cdot y}{\max(|x|,|y|)},$$

where the norms are as in the Jaccard metric. This distance is 1 minus the ratio of the total number of components two odors share to the maximum number of components present in either odor. It satisfies our first criterion, and partially satisfies the second (because if the size of a more complex mixture is increased, the distance between the two odors will increase).

Correlation distances were computed either ‘locally’ per each 100–ms bin (Fig. 5F, S5C), or ‘globally’ by temporally concatenating the binned trajectories (Fig. 5D, S5B). Chance levels for the global measure were computed by shuffling the odor labels of the evoked responses once per trial. For the local measure, response odor labels were shuffled once per bin and per trial (red), or PN identities were shuffled for each odor and each bin, but using the same shuffling across trials for a given odor and bin (black). The figures show means and S.E.M.s across trials.

**PN and KC responsivity**

A PN or KC was classified as responding if its firing behavior during the response window met two independent criteria of response amplitude and reliability, similar to (Mazor and Laurent, 2005).

*Amplitude:* the neuron’s firing rate in the response window (measured in successive 200 ms bins, averaged across all trials) had to exceed the mean baseline and $n$ standard deviations of the baseline rate (measured across 200 ms bins and all 7 trials) in at least one bin within the response window. Baseline rate was measured for each cell–odor pair over a period of 600 ms preceding stimulus onset and over all 7 trials. Values of $n$ of 1.5 or 2 gave low rates of both false positives (during baseline) and false negatives (during stimulation) in PNs. Values of $n$ between 0 and 4 made no significant difference with KCs. We show results with $n = 1.5$.

*Reliability:* to ensure that responses detected were reliable even at low firing rates in KCs, we required that at least one trial more than 50% of all trials (*i.e.*, at least 4/7) with each odor contained at least one spike during the response window. Our metric for responsiveness is extremely conservative, because it measures PN
activity for only 1 s shortly after odor onset. In reality the dynamics of PNs last for as long as 3–4 s after odor offset (e.g., rebound excitation that occurs later in PNs is not captured by our metric).

**ROC Analysis**

We used ROC analysis (Fawcett, 2006) to evaluate individual PNs and KCs as classifiers for the presence of single odor components in mixtures. In the ROC framework, a binary classifier computes a score for each input that is thresholded to assign a class (positive or negative) to the input. The fraction of positive inputs that are correctly classified yields the true positive rate (TPR) of the classifier at the given threshold, while the fraction of negative inputs that are incorrectly classified yields the false positive rate (FPR). Plotting the TPR against the FPR as the threshold is varied yields the *ROC curve*, and the area under this curve is the *AUC* score which is a measure of the performance of the classifier (0 for perfect reverse classification, 0.5 for chance performance, 1 for perfect classification). Applied to the PNs and KCs, we first found cells that responded (using our response criteria above) to at least one of the single odor components. For each single odor component, we partitioned all odor conditions into two classes. For example, for the case of component A, the “positive” class consisted of all odor conditions including A (A–high, A, AB, AC, AD, AX, ABC, ACD, AXZ, ABCD, ABCX, ABCDX, ABCWX, ADWYZ, AWXYZ, ALL) and the “negative” class of all conditions without A (C–high, W–high, B, C, D, W, X, Y, Z, BC, DW, WX, WY, WZ, XY, XZ, YZ, BCX, BDW, DXY, WXY, WYZ, BCWX, BDWX, DWYZ, WXYZ, BCWXZ), not including paraffin oil. We then used ROC analysis to evaluate each responding cell as a classifier for the presence of each of the single odors it responded to (*i.e.* if a cell responded to components A and B, it was evaluated as classifier for A, and again for B), using the total number of spikes produced in the response window across all trials of a given odor as the classifier’s score for the odor. The top panel of Fig. 6E shows some sample ROC curves, and the bottom panel shows the distribution of AUC scores for all cell–odor pairs in the two populations. We repeated the analysis for longer response windows of 1.4 s and 2 s, and for single trials, and observed no significant differences.
To compute the evolution of the AUC distribution over time, we examined the same set of PN– and KC–odor pairs as in the bottom panels of Fig. 6E, but computed the AUC distribution using spike counts in consecutive 50 ms time bins spanning the period [-1 s to 3 s] relative to odor onset, and plotted the median and interquartile range for the two populations in Fig. 6F.

**Mean KC Spike Latency**

Mean KC spike latency was defined as the peak of the KC PSTH computed using a 20 ms Gaussian smoothing kernel, averaged across 7 trials and baseline subtracted.

**Multi-stage Adaptive Lasso**

Lasso regression (Tibshirani, 1996) regularizes ordinary least squares regression by constraining the sum of the absolute value of the weights. Its advantage over ridge regression is that it can shrink weights identically to zero, while ridge-regression only scales them. The adaptive lasso improves on the lasso by penalizing large coefficients less than small ones, and the multi-stage adaptive lasso tries to reduce the probability of including spurious regressors by running the adaptive lasso repeatedly, each time using the previous run's best estimate of the fit coefficients (typically estimated using cross-validation), until the form of the model has stabilized. Our implementation of the adaptive lasso was based heavily on the lasso function included in MATLAB’s Statistics toolbox, and allowed us to provide weights for the coefficients and to limit the weights to be strictly positive by removing them from the active set when they became negative.

**Constructing Single KC Responses from PNs**

To regress a KC’s responses on the PNs, we first computed its mean firing rate in 10 consecutive 100-ms time bins starting 0.1 second after odor onset. We concatenated these responses to form a 440 = 44 odors x 10 bins / odor response vector for the KC. We performed the same procedure for the PNs, and ran the multi-stage adaptive lasso algorithm (Buhlmann, 2011) for 10 stages or until the number of regressors in the model stabilized, whichever came first (with
a non-negativity constraint on the PN weights). We then computed the fraction of the variance left unexplained by the fit (SSE/SST) and the correlation coefficient between the response and the fit—since, due to regularization, the residuals are not necessarily uncorrelated with the fit and SSE does not determine the correlation coefficient, as it does in ordinary least-squares regression.

Shuffled populations of cells were created from un-shuffled ones by reassigning the odor responses ‘whole’ among the cells, i.e., without perturbing their temporal structure either within or across trials. Thus a shuffled cell’s responses to a given odor became another cell’s responses to a different odor.

We measured how well the PNs were suited to reconstructing the recorded KC responses by producing 250 shuffled KC populations using the procedure above, reconstructing them using the un-shuffled PN population, and reported the SSE/SST and correlation coefficient distributions for the resulting over all cells and all shuffles in Fig. 7H. We also measured how well suited the recorded PNs in particular were to reconstructing the KC responses by producing 1000 shuffled PN populations and using them to reconstruct the un-shuffled KCs, while constrained to using the same number of shuffled PNs in each reconstruction as were required when using the un-shuffled PNs. The reconstruction metrics were computed for all KCs and all shuffles and the distributions summarized in Fig. 7H.

**Constructing PN Population Trajectories from KC Responses**

We searched for a fixed basis in which to describe the response of the PN population in any given time bin as a linear combination of basis elements, with the coefficients of combination determined by the KCs (plus a constant term). We can write this as looking for a solution to $U = ZV$, where $U$ is a $\#\ PNs \times \#\ time\ bins$ matrix whose columns contain the PN population responses for each of the 440 time bins formed from 10 response bins for each of 44 odors, the $\#\ KCs \times \#\ time\ bins$ matrix $V$ is the corresponding matrix for the KC responses, and $Z$ is the $\#\ PNs \times \#\ KCs$ matrix whose columns are the basis vectors that each KC corresponds to. While in general an exact solution to the equation above cannot be found, we could ‘settle’ for the least squares solution found using the Moore–Penrose pseudo-inverse: $Z = UV'(VV')^{-1}$. However, the resulting solution could contain many spurious small but non-zero weights, making it harder to interpret
the resulting basis matrix. To remedy this situation, let $Y(Z) = ZV$ be the estimated PN reconstructions for a given basis matrix $Z$. We wanted to find the matrix $Z$ such that the sum of the squared error (SSE) between $U$ and $Y(Z)$ was reduced. Clearly the SSE would be left unchanged if we instead considered $U'$ and $Y(Z)' = V'Z'$. But the columns of $U'$ are just the responses of single PNs over the 440 bins, the columns of $V'$ the corresponding KC responses, and the columns of $Z'$ therefore the reconstruction weights for each PN. Because there is no requisite dependency between the column of $Z'$, they could be treated independently, and we minimized the SSE by setting these columns to minimize the reconstruction error of each PN using the KCs. Hence we applied the multi-stage adaptive lasso algorithm we used to construct KCs from PNs in reverse (and without the constraint on strictly positive weights), to find the columns of $Z'$ one at a time, and transposed the result to get the desired weight basis matrix. Having done so, we computed the average value of the unexplained variance in explaining the PN population response at each of the 10 bins in a given odor response, as well as the corresponding correlation coefficients between the PN population vector and the fits. We plotted the distribution over the 44 odors in Fig. 7K.

Just as when reconstructing single KCs, we then measured how well matched the KCs were to the PNs. First, we produced 100 shuffled KC populations and for each, computed fits to the unshuffled PN responses, computed the fit metrics as before, and plotted them in Fig. 7K. As described above, our fits were computed by reconstructing the single PN responses, and we constrained the reconstructions to require the same number of weights as required by the unshuffled KCs. We also used the unshuffled KCs to reconstruct each of the odor trajectories in 50 shuffled PN populations, computed the fit metrics as before and plotted them in Fig. 7K.

**Population Decoding**

To estimate the information carried by PN and KC ensembles about odor component and identity in single trials, we used a decoding based approach (Hung et al., 2005; Meyers et al., 2008). A linear classifier was provided with spike counts in 4 consecutive bins (25 ms each bin) across all PNs (174) and KCs (209) and computed over 2 s shortly after odor onset. The classifier consisted of a weighted sum of PN or KC inputs. The weights were estimated using
regularized least squares regression (Rifkin et al., 2003). This approach can be thought of as multiple linear regression with a constant term. Multiple linear regression cannot determine the weights unambiguously if the sample matrix is ill conditioned, which is often the case with few trials or few spikes (as with KCs). The formulation for RLSC is below:

\[ \mathbf{w} = \mathbf{X}'(\mathbf{X} \mathbf{X}' + \lambda \mathbf{I})^{-1} \mathbf{y} \]

The \( T \times n \) matrix \( \mathbf{X} \) contains spike counts across all cells; each row is one trial (\( T \) contains \( T/2 \) positive trials and \( T/2 \) negative trials); each column represents spike counts in one cell (\( n \) columns represents \( n \) cells). \( \mathbf{w} \) is the \( n \times 1 \) weight vector, a unique weight for each cell. \( \mathbf{y} \) is the \( T \times 1 \) vector of class labels (+1 and -1). \( \mathbf{I} \) is the \( T \times T \) identity matrix, and \( \lambda \) is the scalar regularizer. The larger \( \lambda \) is, the more constraints are placed on the solution; the smaller \( \lambda \) is, the closer the solution is to multiple linear regression. Even a small value of the regularizer punishes unrealistically large weights and ensures stability. Regularization becomes particularly important when the number of input variables (neurons) outnumbers the number of training examples, as was the case here. There usually is an optimal value for \( \lambda \). We tried values of 0.01, 0.1 and 1 but observed no significant difference. Therefore \( \lambda \) was kept constant at 1 throughout. The number of trials in each class during training was always kept the same to avoid decoding bias. Where the numbers of positive and negative trials were different, we performed 20 or 50 random sub-samples (without replacement) of size equal to the smaller group, from the larger group, to equalize the number of positive and negative trials. The decoding accuracy of the classifier was estimated using cross-validation, as described below for each decoding task.

**Decoding Odor Identity**

To decode odor identity (i.e., which of the 44 odors had been presented), we used all-vs.-all multiclass decoding, one time bin at a time. The number of spikes was counted in each 100 ms time bin sampled at 25 ms intervals with data from each time bin being classified independently, leading to a slight temporal smoothing. Our procedure was as follows. For every time bin \( J \) and every trial \( K \) of every odor \( I \), we built 44x43 binary classifiers (e.g., A vs. Ahigh, A vs. B, A vs. C, A vs. D, A vs. AB, ... Z vs. A, Z vs. AB, etc.). Each classifier \( U \) vs. \( V \) was trained to assign positive valence to the first odor in the pair (e.g. \( U \) in this case), and negative to the second. The data used to train it depended on the identities of \( U, V, \) and \( I \):
1) If neither U nor V were the odor in question I, then all trials for both odors were used to train the classifier.

2) If U = I, then all trials but the K'th of U were used, and all of V.

3) If V = I, then all trials of U were used, and all but the K'th of V. In practice this was done by reversing the signs of the classifier learned in case 2 when V happened to be the positive valence odor.

These classifiers were then tested on the data for trial K of odor I (the withheld trial), the assigned valence computed as the sign of the dot product of the data vector and the learned weight vector (a sign of zero was assigned a random valence) and 44 scores computed: 1 OwnScore, and 43 OtherScores:

- OwnScore: The sum of positive valences assigned by all 43 I vs. V classifiers, i.e. the classifiers trained to give a positive valence to odor I without having seen trial K.

- OtherScores(V): The sum of positive valences assigned by all V vs. U classifiers, where U, V are not equal to I (and thus the classifiers were trained on all trials of both V and U), and the V vs. I classifier (trained on all trials of V and all but the K'th trial of I).

The intuition is that OwnScore should be high because the I vs. V classifiers have been trained to assign positive valence to odor I. OtherScores should all be low, because odor I should not look like the odors they've been trained to assign a positive valence to, and so their valences for the withheld trial of I should be essentially random (for V vs. U, U not equal to I), or negative for (V vs. I).

The trial was recorded as correctly classified if OwnScore > max(OtherScores). Otherwise, it was marked as incorrect if OwnScore < max(OtherScores). If OwnScore = max(OtherScores), the trial was assigned at random to one of odor I or the other odors which achieved the maximum, and marked as correct if this random assignment ended up being to odor I.

In this way we computed an identity decoding score for odor I in time bin J, with the K'th trial removed. We then averaged the result over trials to get the mean accuracy when identifying odor I in time bin J. The mean and S.E.M. of this accuracy over odors for each timebin were reported as the overall identity decoding performance (Fig. 8A and Fig. S8A). Chance performance was 1/44 or 2.27%.
Decoding Odor Category

To decode odor component or category information, we built 8 different classifiers (A vs. not-A, B vs. not-B, C vs. not-C, D vs. not-D, W vs. not-W, X vs. not-X, Y vs. not-Y, Z vs. not-Z), one for each odor component. We selected the odors for each classification task by attempting to satisfy the following two constraints: (1) that the two classes differ only by the odor component (e.g. A, AB, ABC, AC vs. B, C, BC) since the difference between e.g. ABC and WXY is more than just the presence of A, and (2) that the two classes have approximately the same number of $n$-level mixtures for each value of $n$, since there is a positive correlation between the number of KCs activated and the number of odor components in the mixture $n$ (see Fig. S7A). The resulting class partitions for each classifier are listed below.

Classification performance for a given time bin was computed as follows: Since the number of trials available for the two valences were always different, we performed 20 random sub-sampling runs, where in each run the positive and negative trials were each sampled randomly without replacement to equalize the group sizes. In each such run, we looped through each of the trials for each valence, trained the classifier on the remaining trials, and tested it on the withheld trial, yielding a binary (correct/incorrect) performance measure. The performance for the time bin was computed by averaging over all withheld trials (of both valences) in a given sub-sampling run, and over all sub-sampling runs for the time bin. This yielded the time course of mean categorization performance for the given odor component. Overall performance statistics (means, S.E.M.s; Fig. 8B) were then computed over the 8 odor components. For the per-component results (Fig. S7B), the number of sub-sampling runs was increased to 50. Performance in a given time bin was computed by averaging over all withheld trials for a given valence and sub-sampling run. The mean and S.E.M. over valences and sub-sampling runs was reported as the performance measure for the component in Fig. S8B.

Because this classification is binary, chance performance was at 50%.

Decoding Odor Generalization

The procedure for computing odor generalization performance for a given component was similar to that for computing odor categorization performance. The main difference was that the data for one entire odor, rather than just one
trial of an odor, was left out during training. In detail, we looped through each of
the odors in the classification task and removed it from the training set. Then for
each time bin, we performed 20 sub-sampling runs where we sampled the trials
for the remaining odors in the classification task without replacement to equalize
the number of training trials in both valences. A classifier was trained on all of
these trials, and tested on all trials of the withheld odor. Performance for the
withheld odor was defined as the fraction of test trials correctly classified. The
overall performance for the component at that time bin was computed by first
computing the average performance over all sub-sampling runs for all in-group
odors and out-group odors separately, and then averaging across the two
groups. This was done to avoid biasing the performance measure towards the
performance on the out-group odors, of which there were usually a few more
than in-group odors. Overall performance statistics (means, S.E.M.s; Fig. 8C) were
then computed over the 8 odor components. For the per-component results (Fig.
S8C), the number of sub-sampling runs was increased to 50. Performance in a
given time bin was computed by averaging over all withheld odors for a given
valence and sub-sampling run. The mean and S.E.M. over valences and sub-
sampling runs was reported as the overall performance measure for the
component in Fig. S8C.

Because this classification is binary, chance performance was at 50%.

Performance of Random PN Subsets
Our dataset contained a much larger fraction of the total PN population (174/830
= ~21%) than the total KC populations (209/50,000 = ~0.4%). To determine the
effect of this discrepancy on decoding performance, we computed the
identification, categorization, and generalization performance of 100 random
subsets of 8 PNs. We used 8 PNs instead of the 4 that would equalize that
sampling ratios above to allow for the case of each PN encoding one of the 8
mixture components that we used, so as to avoid introducing an artificial bias
against the PNs. We then computed the mean ± S.E.M. of the performance of
each of the PN subsets on each of the tasks just as we did for the full PNs and
KCs populations. We characterized the performance of each subset in a given task
by the maximum value (over time) of its mean accuracy. We then plotted the
results for the subset whose performance was closest to the median in Fig. 8A–C.
Balanced Odor Classes for Decoding Categorization and Generalization

For categorization and generalization, the following odor groupings were used to ensure as much as possible that for each odor $X$ vs. not-$X$ classification task, (a) the number of $n$-level mixtures was the same in both the $X$ and not-$X$ categories, and (b) the odors in the two groups differed only by the presence or absence of the component $X$.

**Odor A vs. not-A**

A: A (4x), A, AB, AC, AD, AX, ABC, ACD, AXZ, ABCD, ABCX, ABCDX, ABCWX, ADWYZ, AWXYZ

A’: C (4x), B, C, D, X, BC, DW, XZ, BCX, BDW, DXY, WXY, WYZ, BCWX, BDWX, DWYZ, WXYZ, BCWXZ

**Odor B vs. not-B**

B: B, AB, BC, ABC, BCX, BDW, ABCD, ABCX, BCWX, BDWX, ABCDX, ABCWX, BCWXZ

B’: A (4x), A, C (4x), C, X, AC, AD, AX, DW, WX, ACD, AXZ, DXY, WXY, DWYZ, WXYZ, ADWYZ, AWXYZ

**Odor C vs. not-C**

C: C (4x), C, AC, BC, ABC, ACD, BCX, ABCD, ABCX, BCWX, ABCDX, ABCWX, BCWXZ

C’: A (4x), W (4x), A, B, X, AB, AD, WX, AXZ, BDW, DXY, BDWX, DWYZ, WXYZ, ADWYZ, AWXYZ

**Odor D vs. not-D**

D: D, AD, DW, ACD, BDW, DXY, ABCD, BDWX, DWYZ, ABCDX, ADWYZ

D’: W (4x), A, B, W, AC, XY, ABC, WXY, WYZ, ABCX, BCWX, WXYZ, ABCWX, AWXYZ, BCWXZ

**Odor W vs. not-W**

W: W (4x), W, DW, WX, WY, WZ, BDW, WXY, WYZ, BCWX, BDWX, DWYZ, WXYZ, ABCWX, BCWXZ
W': A (4x), C (4x), B, D, X, Y, Z, BC, XY, XZ, YZ, ABC, ACD, AXZ, BCX, DXY, ABCD, ABCX, ABCDX

Odor X vs. not-X

X: X, AX, WX, XY, XZ, AXZ, BCX, DXY, WXY, ABCX, BCWX, BDWX, WXYZ, ABCDX, AWXYZ

X': A (4x), C (4x), W (4x), A, D, W, Y, Z, BC, WY, WZ, YZ, ABC, ACD, BDW, WYZ, ABCD, DWYZ, ADWYZ

Odor Y vs. not-Y

Y: Y, WY, XY, YZ, DXY, WXY, WYZ, DWYZ, WXYZ, ADWYZ, AWXYZ

Y': D, W, X, Z, DW, WX, WZ, XZ, AXZ, BDW, BCWX, BDWX, ABCWX, BCWXZ

Odor Z vs. not-Z

Z: Z, WZ, XZ, YZ, AXZ, WYZ, DWYZ, WXYZ, ADWYZ, AWXYZ, BCWXZ

Z': W, X, Y, AX, DW, XY, WY, BDW, WXY, DXY, BCWX, BDWX, ABCDX, ABCWX

SUPPLEMENTAL ANALYSIS

Representations of Binary Mixtures by Single PNs

Figure 2F shows that for most PNs, responses to binary mixtures, when they can be fit at all by the component responses, are usually fit best using only one of the components. These results are shown again in Fig. S2A. Figure S2B indicates the quality of the fits. The matrices are arranged as in Fig. S2A, but colored according to the $R^2$ value of their fits. The two columns in Fig. S2C are arranged as in Fig. 2F, but colored to indicate the signal-to-noise ratio (SNR) of the response, defined as the ratio of the mean squared error when using the baseline mean to predict the response, to the baseline variance (see below).

Comparing Figs. S2A and S2C suggests that non-constant fits were found whenever the response SNR was sufficiently high. Non-constant models fit approximately 62% of responses in the mixture morph experiments overall, but
89% of those for which response SNR > 3 dB (mean energy of deviations from baseline twice that at baseline). The mean ± SEM of $R^2$ was 0.35 ± 0.01 overall, but was 0.53 ± 0.01 when response SNR > 3 dB. Thus in most cases where a PN responded reliably to a binary mixture, the response to that mixture resembled most the response to one (most cases) or both components in a manner that accounted for more than half of the response variance, on average.

Figure S2D shows the distribution of best models for the mixture responses of citral-, octanol-, and mixture-type PNs. For all three response-types, the majority of responses were best fit by scaling the inputs. The mean and standard deviations of the scaling factors were (0.54, 0.26), (0.51, 0.17), and (0.54, 0.18), respectively, with less than 5% of values less than zero or greater than 1. Hence most binary-mixture responses were best fit by scaling one of the components or the sum of the two responses by about one half. We then asked whether mixture composition affects the scaling coefficients. At each dilution, we looked for all citral-type PNs that produced a citral-type response at the dilution in question as well as at cit140:oct30 (the mixture closest to citral), and subtracted the scaling factor of the latter from the former, including unit-type responses with a scaling factor of 1. We repeated this procedure for the octanol type responses. In Fig. S2E, the mean and S.E.M.s of these differences are plotted as a function of dilution. The data have been arranged in opposite order of $x$ for citral– and octanol–type responses (i.e., citral–type values plotted at 140:60 correspond to a cit140:oct60 mixture, while the octanol–type values plotted at the same value of $x$ correspond to cit60:oct140). Significant differences from zero (*, $p<0.05$, paired t-test) occurred for negative values of $y$, indicating increased suppression as the concentration of the complementary odor was increased. An overall trend with mixture dilution was present in both traces, and could be fit with sinusoids (citral: $R^2 = 0.92$, $p < 10^{-5}$; octanol: $R^2 = 0.76$, $p < 10^{-3}$). This analysis shows an increase in suppression of the dominant component response in binary mixture whenever the complementary odor was at an intermediate, lower concentration and a reduction in suppression when the proportions were reversed. These trends probably reflect gain control mechanisms within the antennal lobe.

As shown in Fig. S2D, the majority of binary–mixture responses were best fit by scaling the response to one or the other component; true mixture–type responses were relatively rare. This could have resulted from the suppression of the
response to one of the components when presented in the mixture, indicating a strong nonlinearity. But this result could have other explanations, such as redundancy in the component responses. For example, if a PN responded to both components but to each with very similar response profiles, only one would have been selected to contribute to the mixture fit, causing the other to be artificially eliminated during model selection. We thus computed the 'SNR-angle' of each fit response. The SNRs of the component responses were computed as for the response SNR, substituting the component responses for the mixture response. The resulting two SNR values (in dB) defined a 2D vector whose angle to the x-axis we define as the SNR angle: an angle of zero indicates a response to octanol and no response to citral; an angle of pi/2 indicated the converse. In Fig. S2F we plot a frequency distribution of these angles for the different response types. The majority of octanol–type responses had an SNR angle near zero; conversely, the majority of citral–type responses had an angle of ~pi/2. This indicates that many citral– and octanol–type responses were for cell–mixture pairs in which there was a clear and reliable response to only one component in the mixture. Hence despite the apparent suppressive nonlinearity implied by a single–component fit, the majority of such responses were in conditions where the complementary response was weak, making suppression unnecessary to explain the fit. Hence a simplified description of the above results is that ~80% of the PNs could be split into two groups based on their relative affinity for octanol or citral, and that their responses to the binary mixtures of these components were most simply explained by a scaling of their responses to one of the components.

**Representation of Complex Mixtures by Single PNs**

Just as in the case of binary mixtures, we attempted to describe the responses of single PNs to complex mixtures using their component responses. We found (Fig. 4B) that when responses could be so fit, only a single component was typically required for each PN. Here we examine these results in more detail. Figure S4A repeats the data in Fig. 4B, and Fig. S4B,C show the qualities of the fits. Overall, best fits involving one or several components were found for 31% of the PN–mixture conditions. Comparing Fig. S4A and S4C suggest that such fits were possible whenever the SNR was sufficiently high (61% of PN–mixture conditions in which SNR > 3 dB). The mean ± SEM of R² overall was 0.15 ± 0.0034, but 0.30 ± 0.0050 when SNR > 3 dB. These values are significantly lower than for the binary
mixture experiments (0.35 ± 0.0075, and 0.53 ± 0.0080, respectively, see above). We also compared the results of two sets of separate experiments directly (i.e., based on different PN recordings) at the mixture condition they had in common (cit100:oct100), tested in the binary mixture concentration series experiments and called odor AC in the complex-mixture experiments. In the latter, 21% of PN responses to AC could be fit to a non-constant model (46% of those in which response SNR > 3 dB). In the binary-mixture experiments, 60% of cases of odor AC could be fit overall (85% of those above 3 dB).

To understand the potential source of the discrepancy between the two sets of experiments, we again compared the odor–AC results to those with cit100:oct100 (binary-mixture set). For each PN, we computed the maximum SNR of its component responses, and the SNR of its mixture response. We then categorized the PN responses into four groups based on their component and mixture responses being above or below 3 dB: (1) Silent: component and mixture SNR < 3 dB; (2) Suppression: component SNR > 3 dB, mixture SNR < 3 dB; (3) Emergent: component SNR < 3 dB, mixture SNR > 3 dB; (4) Full response: component and mixture responses > 3 dB. The percentages of PNs with suppression- or emergent-type responses were similar in the binary mixture and complex mixture experiments: 4.2%, and 15% for suppression- and emergent-type responses in the former case, vs. 8.0% and 19% in the latter. A large difference was found in the percentage of silent cells: 24% for cit100:oct100, vs. 47% for odor AC. This suggests that the main reason for an overall lower fraction of responses that could be fit in the complex mixture conditions is that these mixture conditions engaged fewer cells, possibly due to adaptation. Note that this explanation does not account for the lower fraction of responses above 3 dB that could be fit in the complex mixture conditions, which must be due to an increase in gross nonlinearities in the complex mixture conditions. These results will be examined in the Supplementary Discussion.

Note also that some PNs had a secondary best fit. For example, a “C-type” PN had a secondary best fit with component W (a “Cw” type response), and a W-type PN with component C (a “Wc” type response). These components are chemically similar (C = citral, W = isoamyl acetate). Other pairings could also be found, such as Xw, Wb, etc.
Figure S4D shows the distribution of model fits for each response type. The majority of the responses were scaled (57%) and un-lagged (52%). The mean ± S.E.M. of the scaling weights computed over all such PNs for all fits using the preferred component of each was 0.71 ± 0.0094, similar to what was found with binary mixtures when pooling over the preferred-component fits for citral- and octanol-type PNs (0.74 ± 0.0034).

We next looked for systematic trends in the scaling coefficients of the fits with mixture level in those PNs for which one type of response dominated. We computed, for each of these PNs, the average value of the scaling coefficients used at each mixture level in which the best fit was a unit- or scaled-version of the preferred component response. This yielded up to 5 values for each PN: the mean scaling factors at mixture levels 2–5, and 8 (not exactly 5 whenever a PN didn’t have the required type of fit for any of the mixtures at some mixture level). Of the 124 available PNs, we kept the 98 for which at least 3 of the 5 values were available. The data for these PNs are plotted in Fig. S4E according to their “best-fit” odor component; no clear trend could be detected. We then computed the Spearman rank correlation of scaling coefficient with the mixture level for these PNs. For 8 of the 98 PNs there was no change in scaling coefficient with mixture level, and to these we assigned a correlation value of 0. The mean and median of the correlation coefficients were −0.15, and −0.25, respectively, suggesting a slight negative trend, though it was not significant (median not significantly different from zero; p-value = 0.073, sign-test). Hence, PNs whose mixture responses were best fit by the response to one single component responded to mixtures containing that component by scaling the component response to ~3/4 of its unmixed magnitude, on average.

Finally we examined the extent to which a PN’s mixture response best explained by a particular component implied a suppression of the other component responses. For each of the responses that were fit most conservatively by single components (1523 out of 5568), we computed the SNR of the response to the “best” single component, and the maximum SNR of the responses to the other components present in the mixture. We thus positioned each response in 2D space, and computed the SNR angle such that an angle near zero meant that the response to the best single component dominated over all the others, while an angle near pi/2 meant that at least one of the other component responses dominated over the “best” response. Figure S4F plots the distribution of these
angles (blue). Note a clear peak near 0, and only a small one near pi/2. However, there is a large secondary peak near pi/4, suggesting that in many of the responses fit by a single component, at least one secondary component was suppressed, or had a response time course sufficiently similar to the preferred component that its contribution to the fit was minimal. Limiting the analysis to the 901 responses from “A- to Z-type” PNs that were fit by their best-components (i.e. using only the A-component fits for A-type PNs, etc., ignoring the occasional fits that used other components) slightly increased the height of the peak at zero (red curve), reduced the one at pi/2, but did not alter that at pi/4. The best component was dominant (|SNR angle|<pi/8) in only about half of the responses (43% overall, 50% for preferred-component responses). These values are lower than those (66%) for the binary-mixture experiments, indicating that component suppression (or redundancy) is greatly increased in the presence of complex mixtures.

The SNR angle cannot distinguish between a secondary component response that is not included in the fit because it appears redundant (e.g., present but similar to and overlapping with the response to the primary component), and one that is actively suppressed. To make this distinction we examined all 339 cases in which |SNR angle – pi/4| < pi/8 , i.e., those for which the secondary response was of similar magnitude to that the primary. For each of these cases, we recomputed the fit but using only the secondary component response. We reasoned that if the secondary response was indeed redundant, the coefficient of this new fit would be similar to the first one, while if it was being actively suppressed, its coefficient would be lower, and even negative. In Fig. S4G we plot the distribution of the ratio of the weight for the fit using the secondary component to that using the primary component. Values range from −2.0, indicating suppression, to ~3, indicating redundancy. We took the threshold between suppression and redundancy to be the dashed line at a ratio of ~0.2 because manual inspection of the fits showed that when the ratio was small but positive, the best fit was essentially a constant function. Forty eight percent of the fits (11% of all single-component fits) were below this threshold, indicating an active suppression of the secondary component. The mean ± S.E.M. of the correlation coefficient between the primary and secondary component responses in these cases was −0.040 ± 0.014, and −0.096 ± 0.011 between the secondary component and the mixture response. For the 52% of cases (12% of all single-component fits) in which the
responses were above threshold (i.e., redundant), the mean ± S.E.M. was 0.41 ± 0.017, and 0.35 ± 0.013 between the second component and the mixture response. Thus, when the fit considering a secondary component required only a moderate scaling, that component response was positively correlated with that to the primary component; conversely, when the fit required a suppression or subtraction of the secondary component response, the correlation was weak and/or negative, as should be expected.

In summary, our results suggest that 124/174 = 71% of the PN population can be split into 8 groups based on which single component response explained most conservatively the majority of the mixture responses. The average fit was a scaling of the component response by a factor of ~3/4, which did not vary much with mixture complexity. PNs varied in their dilution sensitivity, from those responding when only one other component was present in mixture, to those responding to all mixtures containing their “best” component. Responses requiring more than one component existed but were rare; several PNs did however exhibit a secondary odor “preference”. In about half of the single-component fits in which the PNs’ “preferred” component was used, the response to the “best” component dominated the others. In about 40% of cases a second component response was also present but was absent in the fit because it was redundant with the single component response (~1/2 of the time), or because it was weakly correlated with the mixture response (~1/2 of the time). Fewer responses could be fit than in the binary mixtures experiments, partly due to there being fewer strong responses overall, but also due to increased nonlinearity in the mixture responses.

**Distribution of Component Sensitivity in the Antennal Lobe**

Our results suggest that component sensitivity in the antennal lobe is distributed among the PNs. How could this be accomplished? One possibility is that sensitivity to components is built-in, but our data argues against this. One of the odors conditions, cit100:oct100 was present in both the binary mixtures and the complex mixtures experiments. If component sensitivity were built in, and because PN sampling was performed in the same, unbiased manner in both sets of experiments, we would have expected to observe approximately the same number of fits to this mixture response in both sets of experiments. Rather, fits
were found for three times as many mixture responses in the binary morph experiments as in the complex–mixture experiments. A possible explanation is that the antennal lobe tuned its sensitivity to the odors encountered most often during the course of each experiment. The fact that we found more PNs sensitive to the component most commonly used in mixtures (odor X, 2,3-butanedione) is consistent with this explanation. This observation could have important general consequences for the design and interpretation of all experiments.

Conversely, the fraction of PNs none of whose mixture responses could be fit was about the same in both sets of experiments (binary mixtures: 24/168 = ~14%; complex mixtures: 34/174 = ~20%; pooled: 58/342 = ~17%). These PNs may have been sensitive to a different set of components than those tested. Incorporating this non-responsive population, and assuming that the sensitivity of the remaining PNs is split evenly between the 8 components tested allows us to predict the number of mixture responses that could be fit. Splitting the PNs among the 8 components used in the mixture experiments, our model would predict that a fraction \((1 - 0.17) \frac{m}{8}\) of responses could be fit. For the two-component mixture cit100:oct100, this predicts that \(0.83 \times \frac{2}{8} \times 100\% = \sim21\%\) of responses could be fitted using the responses to the components; this is indeed the observed fraction. Averaged over all mixtures, this predicts that \sim34\% of responses could be fitted, a value close to the observed one (31\%). Thus our results suggest a first-order approximation of the antennal lobe in which component sensitivity is distributed adaptively (i.e., based on immediate or recent experience) among the PNs and in which PNs will respond within a cell-specific range of dilutions to their “preferred” component.

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