

Decoding of Context-Dependent Olfactory Behavior in *Drosophila*

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SUMMARY

Odor information is encoded in the activity of a population of glomeruli in the primary olfactory center. However, how this information is decoded in the brain remains elusive. Here, we address this question in *Drosophila* by combining neuronal imaging and tracking of innate behavioral responses. We find that the behavior is accurately predicted by a model summing normalized glomerular responses, in which each glomerulus contributes a specific, small amount to odor preference. This model is further supported by targeted manipulations of glomerular input, which biased the behavior. Additionally, we observe that relative odor preference changes and can even switch depending on the context, an effect correctly predicted by our normalization model. Our results indicate that olfactory information is decoded from the pooled activity of a glomerular repertoire and demonstrate the ability of the olfactory system to adapt to the statistics of its environment.

INTRODUCTION

A prominent feature of the olfactory system is its widely distributed code. Even monomolecular odorants typically bind to multiple types of olfactory receptors (Malnic et al., 1999), recruiting multiple postsynaptic glomeruli without apparent spatial or chemotopic organization. Different odorants recruit different sets of glomeruli (Friedrich and Korsching, 1997; Hallem and Carlson, 2006; Joerges et al., 1997; Ng et al., 2002; Rubin and Katz, 1999; Uchida et al., 2000; Wang et al., 2003) with distinct temporal dynamics (Cury and Uchida, 2010; Friedrich and Laurent, 2001; Spors and Grinvald, 2002; Wehr and Laurent, 1996), and most glomeruli respond to a wide array of odors. These observations demonstrate that in a range of organisms, including insects, fish, and mammals, early olfactory information is represented as spatio-temporal patterns of glomerular activity. How these activity patterns are decoded by the brain to guide odor-evoked behavior, however, remains largely unknown.

Olfactory research in *Drosophila melanogaster* has provided elements of answer to this question. Using behavioral genetics, attraction, and aversion to specific odors have been linked to the activation of one or a few glomeruli in the antennal lobe

(AL) (Ai et al., 2010; Dweck et al., 2013; Min et al., 2013; Rondros et al., 2014; Schlieff and Wilson, 2007; Semmelhack and Wang, 2009; Stensmyr et al., 2012; Suh et al., 2004). These observations suggest a labeled-line coding strategy, in which individual glomeruli convey signals of specific ethological relevance for the animal and their activation triggers the execution of hard-wired behavioral programs. However, it remains uncertain whether this is a property of a specialized subset of glomeruli or constitutes a general coding principle in the AL. More importantly, how compound signals from multiple glomeruli are integrated to determine the valence of odors, including mixtures of odors commonly found in natural environments, is poorly understood (Kreher et al., 2008).

Another open question is the coding beyond absolute odor valence. Flies, for example, choose the less aversive odor when forced to make a choice between two inherently aversive stimuli (Tully and Quinn, 1985). Moreover, studies in humans have shown that the evaluation of relative odor valence changes depending on the context (Clepce et al., 2014). How relative valence is computed in the brain and how it is modulated according to the context remains elusive, although a recent study showed that divisive normalization can explain context-dependent choice behavior in primates (Louie et al., 2013).

Here, we used a combination of optical imaging and behavioral techniques to address these questions systematically. We used two-photon imaging to monitor Ca^{2+} signals in the whole AL in response to a diverse set of odors. Comparing these data with odor-evoked behavioral responses allowed us to formulate a decoding model describing how innate olfactory behavior is dictated by the population of projection neurons (PNs), the AL output neurons, in a quantitative manner. We found that a weighted sum of normalized PN responses can recapitulate observed behavior and predict responses to novel odors, including mixtures and odors at different concentrations. The valence assigned to individual glomeruli in our analysis is consistent with previous findings; however, the contribution of individual glomeruli to the behavioral output is small, indicating that odor valence is not dominated by a few privileged glomeruli but depends on pooling small contributions over a large number of glomeruli. This conclusion is further supported by genetic silencing and optogenetic activation of individual olfactory receptor neuron (ORN) types, which evoked modest biases in behavior in accordance with the model predictions. Strikingly, behavioral responses were altered when the same odors were tested in different olfactory contexts. Our decoding model captured this adaptive behavior and correctly predicted that the relative preference of pairs of odors could switch depending

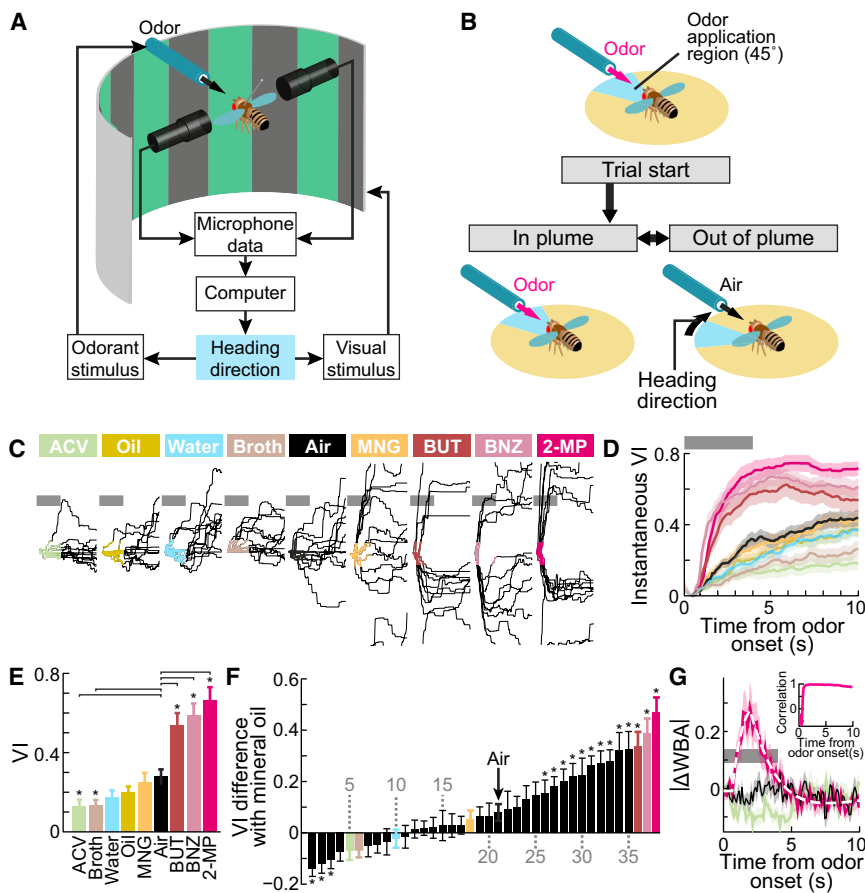


Figure 1. Differential Responses to Attractive and Aversive Odors in a Flight Arena

(A) Schematic of the virtual-flight arena. Flight behavior is probed using microphones, whose output is fed to a closed-loop feedback system that updates visual and olfactory stimuli in real time.

(B) Schematic of the behavioral assay. The fly starts each trial in the center of the odor plume, after which it is free to adjust its heading direction to navigate in or out of the plume.

(C) Trajectories of a representative fly's orientation in the flight simulator in response to 15 repeated applications of six odors and three control stimuli (mineral oil, water, and air). Gray bar indicates the period of odor delivery (4 s). Colored portions indicate that the animal is navigating in the plume. Attractive odors (leftmost) evoke little turning responses in contrast with aversive odors (rightmost), for which sharp turns are observed in the majority of trials. ACV, apple cider vinegar; MNG, mango mimic; BUT, butanal; BNZ, benzaldehyde; 2-MP, 2-methylphenol.

(D) Instantaneous value of the valence index (VI, the proportion of time spent outside the odor plume) plotted relative to the onset of odor application for the nine stimuli in (C). Shaded regions denote SEM computed over flies ($n = 20$). VI values are best separated near the end of the odor application period (horizontal gray bar).

(E) Mean VI values computed by averaging the instantaneous VI in the last 1 s of odor application. Error bars indicate SEM over tested flies ($n = 20$). Asterisks denote statistically significant differences compared with the air control in black (Wilcoxon signed-rank test, Bonferroni-corrected, $p < 0.05$). Mineral oil and water evoke mildly attractive responses compared to air.

(F) Mean VI values for 36 pure odorants and 3 control stimuli ($n = 20$ – 23 flies for each stimulus), relative to mineral oil, in increasing order. Error bars indicate SEM. Asterisks denote stimuli significantly more attractive (3) or aversive (13) than mineral oil (Wilcoxon signed-rank test, Bonferroni-corrected, $p < 0.05$). Colored stimuli are common with (C)–(E); identity of other stimuli is listed in Table S1.

(G) Absolute difference in left and right wing beat amplitudes (mean over all trials and flies) against time from odor onset, for the most attractive (green, ACV) and the most aversive (magenta, 2-MP) odors in (E) and air (black). Aversive response is fitted with a sum of exponentials (dashed line). Shaded regions denote SEM over flies ($n = 20$). Inset: Pearson correlation between the VI and cumulative $|\Delta WBA|$, for odor set in (E).

on the olfactory context. These results highlight the ability of the olfactory system to adapt to the statistics of its olfactory environment, similarly to the visual and auditory systems (Kohn, 2007; Wark et al., 2007).

RESULTS

Differential Responses to Attractive and Aversive Odors in a Flight Arena

We first sought to characterize odor-evoked behavioral responses by monitoring the behavior of tethered flies in a flight-simulator arena. The arena was equipped with a closed-loop feedback system that allowed animals to virtually adjust their heading direction by modulating the amplitudes of their left and right wing beats (Figures 1A and S1). A change in heading direction was accompanied by a corresponding rotation of the visual and olfactory landscapes, defining a virtual-reality-like environment for the animal to navigate. Olfactory stimuli were delivered in the form of a plume, spanning 45° in azimuth, which

flies were free to exit or re-enter by adjusting their heading direction (Figure 1B). The valence of olfactory stimuli in this setup could thus be assessed by monitoring the tendency to navigate in or out of the odor plume.

We initially characterized odor-evoked behavior in this assay using a set of six odorants, which we expected on the basis of prior reports (Knaden et al., 2012; Semmelhack and Wang, 2009) and preliminary experiments to be either strongly attractive or aversive, and three control stimuli (mineral oil, water, and air). Flies often exited aversive odors by producing a fast saccadic turn (Figure 1C). Similar avoidance behavior was not observed in response to air, for which trajectories only showed occasional low-amplitude saccades. Attractive odors, on the other hand, evoked a reduction in turning rate and a tendency to navigate in the odor for a longer time.

To quantify these responses, we defined a measure of olfactory preference termed valence index (VI) as the proportion of time spent outside the odor plume. When computed relative to the time of odor application, instantaneous VIs increased

monotonically and showed consistent trends extending beyond the period of odor application (Figure 1D). Given that attractive and aversive VIs were best separated near the end of the odor application period, we scored each odor using the mean VI value in the last 1 s of odor application (Figure 1E; our results are largely independent of the choice of the scoring period, see Figure S1).

Of the six tested odors, two attractive and three aversive odors evoked significant responses compared with air (Figure 1E). Solvents also evoked mildly attractive responses. Attraction to humidified air has been documented and is strongly reduced by the mutation of Orco (Lin et al., 2015). Attraction to mineral oil also likely depends on the olfactory system, as mineral oil evoked sparse but significant glomerular activity in our measurements (see Figure 2D), and blocking synaptic transmission in Orco-dependent ORNs evoked a small increase in VI (Figure S2).

To further examine responses to diverse odors, we screened odor-evoked responses to a panel of 84 odors, consisting of 36 pure odorants, 4 of which were sampled at 4 different concentrations, and 36 binary mixtures of attractive and aversive odors at various ratios (Table S1). Odors were sampled in sets of 6 and delivered sequentially in each experiment. To verify that the baseline behavior was consistent across different odor sets, we included mineral oil in every experiment. VI values for the 36 pure odorants showed a nearly continuous array of responses (Figure 1F). After correcting for multiple comparisons, 3 odorants were significantly more attractive, and 13 were more aversive, than mineral oil. When compared to air, odors were almost symmetrically split into two sides (Figure 1F). These results demonstrate that flies exhibit both attraction and aversion in response to a variety of odors in our assay.

Fast Escape Responses to Aversive Olfactory Stimuli

Inspection of individual flight trajectories (Figure 1C) revealed that escape responses to aversive stimuli can be remarkably fast. Because flight orientation is controlled by the relative amplitude of wing beats, we computed the absolute difference between the left and the right wing-beat amplitudes (Δ WBA). As expected, air did not evoke noticeable changes in Δ WBA (Figure 1G). In contrast, aversive odors evoked a sharp, transient increase in Δ WBA, whereas attractive odors evoked a slower, sustained decrease. VI values were tightly correlated with the cumulative Δ WBA (Figure 1G, inset), confirming that our behavioral measure reflects an active, stimulus-evoked modulation of the turning rate.

To estimate the response latency, we fitted Δ WBA for an aversive odor with a sum of exponentials (Figure 1G). The fitted curve deflected (10% rise time) 200 ms after the first contact with the odor, indicating that the time required to perceive, process, and respond to an aversive stimulus is shorter than 200 ms.

In Vivo Ca^{2+} Imaging of PN Responses in 37 Identified Glomeruli

We next examined PN activity in response to the same panel of odors under identical odor delivery conditions. In the fly AL, inputs from ORNs are segregated into ~ 50 glomeruli, all of which are identifiable across individuals (Couto et al., 2005; Fishilevich

and Vosshall, 2005; Vosshall et al., 2000). Because PNs in the same glomerulus transmit highly correlated information (Kazama and Wilson, 2009), each glomerulus can be considered as a unit of output. Therefore, the entire olfactory information transmitted from the AL can be obtained by monitoring PN activity in a mere ~ 50 dimensional space. To achieve this, we expressed the calcium sensor GCaMP6f (Chen et al., 2013) under the control of *NP225-Gal4* (Tanaka et al., 2012; Thum et al., 2007), which labels 37 glomeruli with high specificity (Figure 2A). GCaMP fluorescence in the entire AL was imaged at ~ 1.7 Hz using two-photon microscopy in response to the 84 odors in our dataset. To account for brain movement during recordings and variability in AL morphology across animals, we registered all images to a template AL (Figure 2B), and the fluorescence in each glomerulus was extracted by averaging over the spatial extent of the corresponding glomerulus in the template (Figure S3). Consistent with previous reports, glomeruli exhibited various tuning breadths: a glomerulus responded on average to $22\% \pm 16\%$ (mean $\pm$ SD) of all tested odors. Similarly, odors recruited on average 8 ± 5 of the 37 glomeruli (Figures 2C and 2D).

Odor Onset Responses Carry Enough Information to Classify Odors

The rapidity of aversive responses suggests that animals rely on the instantaneous, spatial dimension of PN responses to make perceptual decisions. This requires that a single “snapshot” of the PN response contain sufficient information to categorize odorants. To verify this, we trained a linear classifier to predict odor identity based on PN responses in a single imaging frame. Because each odor was presented four times in each animal, we used data from three trials to train the classifier and the remaining trial to test classification performance. As expected, classification accuracy was close to chance level before odor application, and increased shortly after odor onset (~ 150 ms after odor contact; Figure 2E), peaking near 65% over time (~ 1.3 – 3.7 s after odor contact). It is worth noting that the onset of this increase in classification accuracy matches well with the onset of the aversive response. Performance decayed after odor offset; however, it remained well above chance level throughout the recording period (13 s beyond odor offset), suggesting that residual PN responses carry a significant amount of information about past stimuli.

Responses to Binary Mixtures Suggest Linear Integration of Olfactory Channels

Comparing behavioral and imaging data showed that PN responses in a number of glomeruli exhibited significant linear correlations with VI (Figure S4), suggesting some degree of linearity in the transformation from PN responses to behavior. The total AL activity, however, only correlated weakly with behavior (Figure S4), indicating that the intensity of AL activation is not the sole determinant of odor valence. To examine the relationship in more detail, we focused on mixture data and asked whether behavioral and physiological responses vary in proportion to the mixing ratio of the components. VI scaled linearly with the mixing ratio (Figure 3A). This trend was paralleled in PN activity: linearly regressing the vectors of PN responses on the mixing ratio lead to a very accurate description of PN data (R^2 of fit

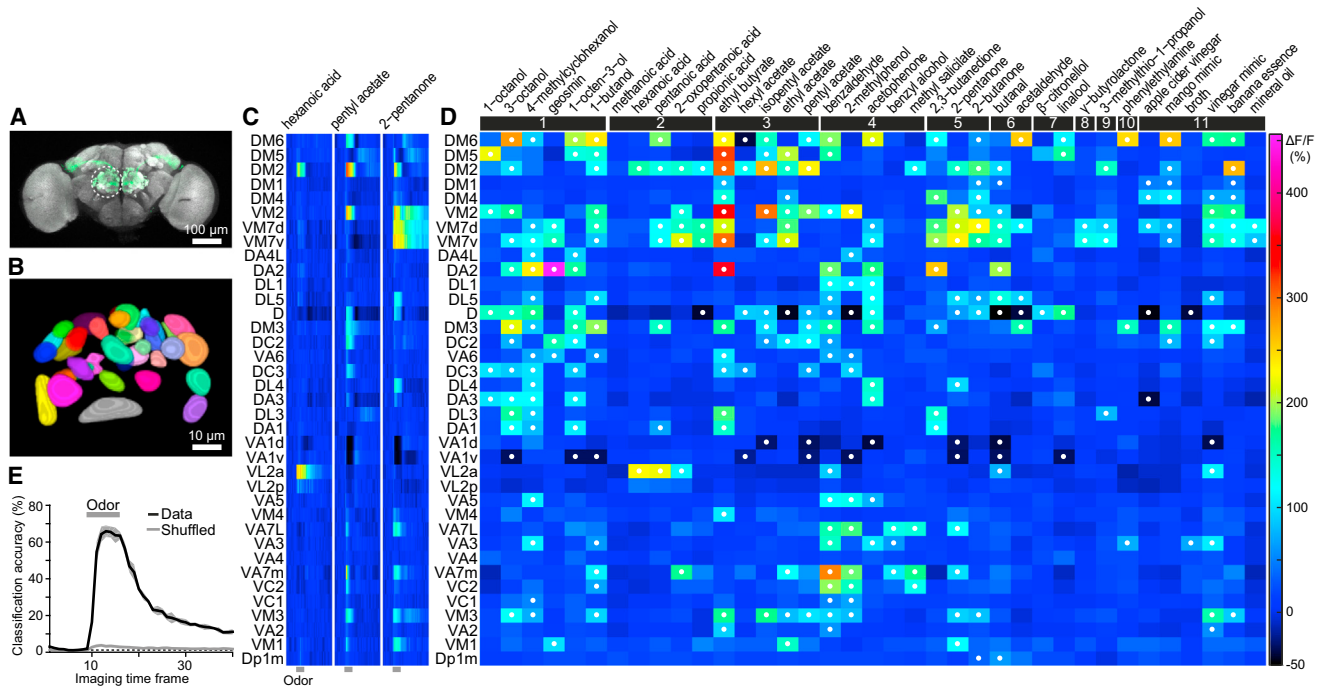


Figure 2. Odor Representations in the Antennal Lobe PNs

(A) Expression pattern of *NP225-Gal4* in the adult brain (*NP225-Gal4/UAS-mCD8::GFP*). The brain was stained with nc82 (gray) and anti-GFP (green). White circle indicates the region scanned in imaging experiments (right antennal lobe).
 (B) Template antennal lobe used in image registration, showing the spatial arrangement of 37 glomeruli labeled by *NP225-Gal4* (arbitrary color code).
 (C) Sample Ca^{2+} responses to three odors. $\Delta F/F$ of GCaMP6f fluorescence is color coded according to the scale bar in (D). Horizontal axis is time (40 595-ms frames), vertical axis is glomerulus. Gray solid bar at the bottom indicates the period of odor application (4 s).
 (D) Mean Ca^{2+} responses to 36 pure odors and the mineral oil solvent (imaging frames 10–17, $n = 4$ –9 brains). Odors are arranged by functional groups: 1, alcohols; 2, acids; 3, esters; 4, aromatics; 5, ketones; 6, aldehydes; 7, terpenes; 8, lactones; 9, sulfur compounds; 10, amines; 11, multimolecular compounds. Significant responses are marked with a white dot (Mann-Whitney U test, $p < 0.01$, see [Supplemental Experimental Procedures](#)).
 (E) Odor classification performance as a function of imaging time frame. The task was to classify 84 odors given the response of 37 glomeruli in a single imaging frame. Shaded region shows SD over four classifiers, each of which uses a different imaging trial as the test. Gray line shows performance after shuffling glomerulus labels. Dotted line indicates chance level (1/84).

0.6–0.97; [Figure 3B](#)). These results suggest a linear relationship between PN responses and behavior.

Olfactory Contrast Normalization Enables Accurate Decoding of PN Responses

Linear models are widely used to examine the relationships between neuronal activity and behavior. In larval *Drosophila*, a linear model based on the activity of ORNs was successful in predicting behavioral responses to a panel of 26 odorants ([Kreher et al., 2008](#)). To test whether this framework could explain our data quantitatively, we constructed a decoding model (referred to as linear model) in which the VI is calculated as a weighted sum of glomerular activity ([Figure 4A](#)). We fit the model parameters using regularized linear regression (see [Experimental Procedures](#)) with training data, and examined its predictive power with test data not used in the fitting procedure. Prediction performance was evaluated using the R^2 coefficient (see [Experimental Procedures](#)). Unexpectedly, we found that while the model could accurately describe the training data, it performed poorly at predicting the test data ([Figure 4B](#)).

We reasoned that a possible cause for the failure of the linear model is that odorants were delivered in sets of six in our experiments: the response to one of the odors in the set may be affected by the experience of the other five odors, for example, through sensory adaptation. To test this, we constructed a second decoding model (referred to as normalization model) in which glomerular outputs were passed through a nonlinear normalization step before the summation ([Figure 4A](#)). Normalization is meant to represent adaptation to the statistics of the olfactory environment and was minimally modeled using a mean subtraction followed by a rescaling by the SD of each glomerulus' response to the odor set, both of which commonly describe neuronal adaptation ([Baccus and Meister, 2002](#); [Chander and Chichilnisky, 2001](#); [Nagel and Doupe, 2006](#); [Ohzawa et al., 1985](#)). Importantly, this form of normalization is compatible with the linearity observed in response to mixtures.

We fit the model parameters using the same procedure as for the linear model. Notably, we found that the normalization model not only accurately described the training data, but also yielded accurate predictions of the test data ([Figure 4B](#)). This prediction performance was highly significant (permutation test, $p < 0.002$),

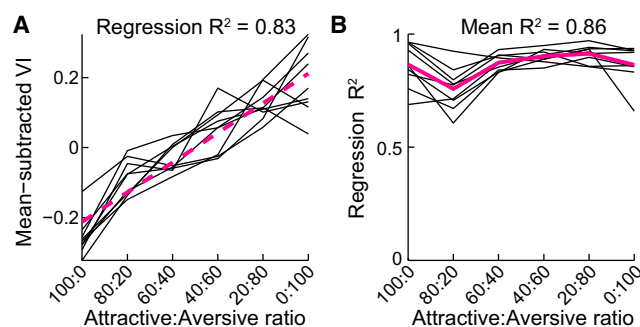


Figure 3. Linearity of Neuronal and Behavioral Responses to Binary Mixtures of Attractive and Aversive Odors

(A) VI (mean subtracted) in response to mixtures of increasingly aversive content, for nine different binary mixtures ($n = 20$ – 27 flies for each mixture) of three attractive and three aversive odors (Table S1). Magenta dashed line is a linear fit to the data.

(B) Linearity of PN responses assessed by fitting the six 37-dimensional vectors of PN responses to each binary mixture set using linear regression. The R^2 coefficient is shown as a function of mixing ratio for each of the nine mixture sets (black lines). Magenta line is the average R^2 over the nine mixtures. Mean R^2 across mixture ratios is 0.86.

suggesting that the normalization operation renders the relationship between the predictors (glomerular activation) and the dependent variable (VI) more linear.

We next tested how the performance varied with the number of glomeruli included in the list of predictors. Because sampling all possible subsets of 37 glomeruli would be computationally prohibitive, we tested instead a multitude (742,700) of randomly chosen subsets. The average prediction performance increased monotonically with the number of glomeruli (Figure 4C, top graph). Importantly, even when restricted to only the best subset of each size (Figure 4C, bottom graph; see Supplemental Experimental Procedures for definition of best subsets), the performance continuously increased up to intermediate sizes (~ 20 glomeruli) before slight overfitting occurred. These results suggest that the majority of glomeruli carry information about the dependent variable.

A final model was constructed by averaging the sets of glomerulus weights obtained in the cross-validation procedure. This resulted in a balanced number of 18 attractive and 19 aversive glomeruli (Figure 4D). We confirmed that the model accurately explained the responses to pure odors and mixtures (Figure 4E), and further challenged the model with an additional set of test data consisting of four odors at three different concentrations. Again, while the linear model failed to predict these data quantitatively, the normalization model gave accurate predictions (Figure 4E).

Silencing Subsets of Olfactory Receptor Neurons Weakly Biases Behavioral Output

These results demonstrate strong correlation, but not causality, between glomerular response and behavioral output. To test the causal relationship, it is necessary to perturb glomerular activity and assess the impact of the manipulation on the behavior. To achieve this in a highly glomerulus-specific manner, we genetically silenced synaptic input to glomeruli by expressing tetanus toxin

(TNT), a potent blocker of vesicular release (Sweeney et al., 1995), in ORNs using Or-Gal4 drivers, which drive virtually no expression outside the target ORNs (Couto et al., 2005; Fishilevich and Vosshall, 2005). We targeted five different glomeruli: DM1, DM4, DM5, DC3, and VC1. These particular glomeruli were chosen in part on the basis of their established attractive or aversive role, and partly based on the predictions of our model. In an attempt to evoke larger effects, we tested two lines in which TNT was co-expressed in three different ORN types. A first set (glomerulus DM5, DC3, and VC1) was chosen to evoke changes toward more attractive VI values and a second set (glomerulus DM4, VA2, and VA4) was selected to induce more aversive responses.

A number of results supported the conclusion that behavior is determined by the summed activity of many glomeruli. First, in accordance with the prediction that blocking input to a small number of glomeruli should have limited behavioral effect, VIs in flies expressing TNT in one or three glomeruli were not substantially different from those in the control flies (Figure 5A). Notably, blockade of synaptic transmission in DM1 or DM5 ORNs, which abolished attraction or aversion to different concentrations of apple cider vinegar in walking flies (Sammelhack and Wang, 2009), did not remove innate responses to this as well as other odors in our experiments (Figure 5A). To rule out the possibility of an incomplete effect of TNT, we expressed TNT in either DM1 or DM5 ORNs while expressing GCaMP3 (Tian et al., 2009) in PNs under the control of *GH146-QF* (Potter et al., 2010) and quantified the change in postsynaptic PN responses to ethyl acetate, an odor that activates both types of ORNs. PN activity in glomerulus DM5 was completely abolished when TNT was expressed in presynaptic ORNs (Figure 5B). Thus, the limited behavioral effect of ORN blockade cannot be attributed to an incomplete action of TNT at least in the case of DM5. In glomerulus DM1, the reduction in PN response was only $\sim 40\%$ (Figure 5B); however, this residual activity is likely to be the result of lateral interactions between glomeruli, which provide excitatory drive to PNs whose cognate ORNs are silenced (Olsen et al., 2007; Root et al., 2007; Shang et al., 2007). To examine this possibility, we inferred a PN-PN connectivity matrix using the correlation structure of Ca^{2+} signals during the baseline period (Figure S5 and Experimental Procedures) and used the resulting connectivity to estimate odor-evoked PN activity in TNT-positive flies. This model accurately predicted both the moderate decrease in DM1 and the strong decrease in DM5 activity in the absence of direct ORN input (Figure 5C). These results support the conclusion that olfactory behavior during flight is not dictated by a few privileged glomeruli.

Second, a closer inspection revealed that VIs were biased in a direction consistent with the weight of each glomerulus in our model. When we computed the correlation between the estimated TNT-induced change in PN activity and the change in VI, the sign of the correlation matched the expected sign in all cases (Figure 5D). Interestingly, synaptic blockade in DM4 and VC1 had an effect on behavior, which was never reported previously, thus expanding the list of contributing glomeruli.

Finally, and most critically, our model quantitatively predicted the behavioral effect of blocking ORNs. We computed the R^2 values between the observed TNT-induced changes in VI and model predictions for single- and triple-ORN blocking

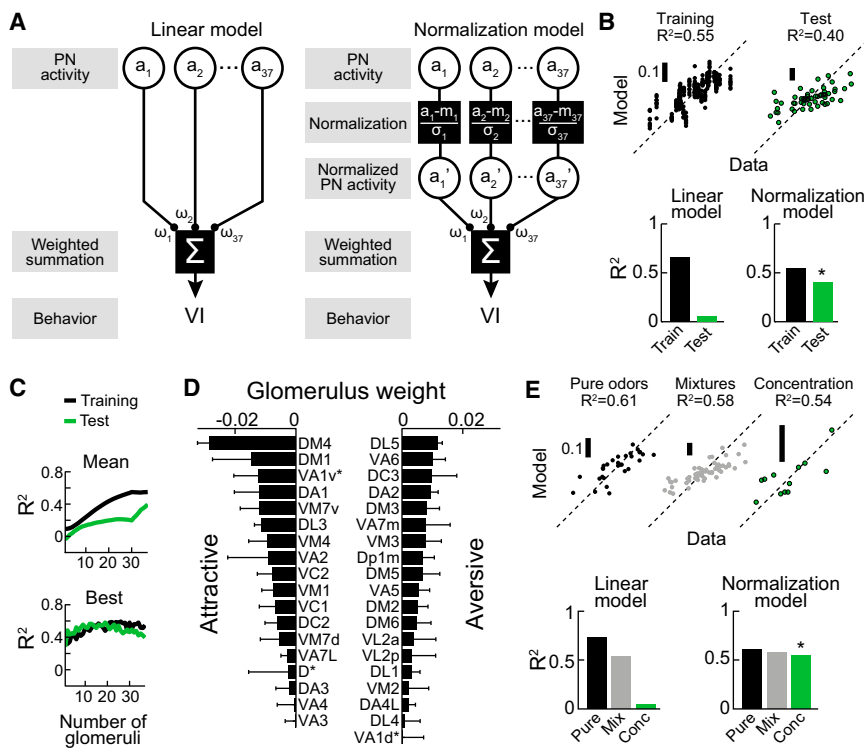


Figure 4. Olfactory Normalization Enables Predictive Decoding of PN Responses

(A) Schematic of the linear and the normalization models. In the normalization model, individual PN responses are normalized, weighted, and summed to produce the estimate of the VI. Normalization parameters (mean, m and standard deviation, σ) are computed using the PN responses to the six odors in each set.

(B) Prediction performance of the linear and the normalization models. Both models are fitted on training data and evaluated on test data using the same procedure. Data comprise 36 pure odorants and 36 binary mixtures ($n = 20$ –27 flies for each stimulus). Top: scatter plots of the normalization model predictions against measured VI. Data from nine cross-validation rounds is pooled. Bottom: summary of prediction performance for the two models. Although the linear model accurately describes the training set, it fails to predict the test set accurately. The normalization model yields accurate predictions that were highly significant in a permutation test ($p < 0.002$).

(C) Prediction performance of the normalization model as a function of the number of glomeruli included in the model. 742,700 randomly drawn subsets of glomeruli were sampled and model parameters were fit as in (B). Top graph shows average performance; bottom graph shows performance of the best subset of each size (see Supplemental Experimental Procedures).

(D) Glomerulus weights in the final model (average over the nine sets of regression weights obtained in cross-validation). Negative weights correspond to attractive and positive weights to aversive glomeruli. Error bars show SD; error bars extending beyond zero have been truncated. Asterisks indicate glomeruli for which the response to more than half of the sampled odors was inhibitory.

(E) Performance of the final model constructed using the weights in (D). Top: scatter plots of the normalization model predictions against measured VI, for the two sets used in cross-validation (pure odors and mixtures) and an additional set of four odors at three concentrations (Table S1). Bottom: a linear model created using the same procedure accurately describes the pure odor and mixture datasets but fails to correctly predict the concentration data. The normalization model yields highly accurate predictions ($p < 0.008$, permutation test). Mix, mixtures; Conc, concentration.

experiments. For both datasets, the R^2 coefficient showed a large increase during the odor-application period (Figure 5E), which was significant (permutation test, $p < 0.006$). The quality of model predictions was higher when three ORN types were manipulated, matching the idea that blocking multiple ORNs evokes larger changes in behavior (Figure 5F). In triple-ORN blocking experiments, performance was maximal when all three glomeruli were blocked in the model, with each individual glomerulus explaining a smaller amount of variance in the data (Figure 5G). This indicates an additive effect of blocking multiple ORN types concurrently. Taken together, these results suggest that blocking ORN biases behavioral output, in a direction determined by the identity of the blocked ORNs, and with amplitude that scales with the change in PN activity in their cognate glomeruli.

Activation of Specific ORNs Evokes Attraction

To further test the causal relationship between glomerular activity and behavior, we asked whether we can bias the behavior according to the model by artificially activating a specific glomerulus. This was achieved by expressing Chrimson, a red-shifted variant of channelrhodopsin (Klapoetke et al., 2014) in ORNs belonging to a particular glomerulus and exciting it with light. We chose to focus on DM4 because this glomerulus had the largest weight in our decoding model. The effectiveness of

Chrimson was verified by recording from antennal ab2 sensilla, which house the DM4 ORNs (ab2A neurons). Application of 637 nm laser light evoked robust increases in firing frequency in ab2A neurons that scaled with light intensity (Figure 6A). We then tested whether light-induced activation of these ORNs had an impact on the behavioral response to odors. We tested two odors, γ -butyrolactone and linalool, which evoke relatively sparse PN activity but excite ab2A neurons. Both odors were significantly more attractive when paired with light than when applied alone (Figure 6B). In contrast, control flies showed no significant change in VI. To further confirm that this increase in attraction is not an artifact of the laser stimulation, we restricted light application to the antennae by covering the eyes and the head cuticle with aluminum foil, so that only the antennae were exposed to the light (Figure 6C). Flies prepared in this way showed a significant decrease in VI in response to light stimulation in the absence of odor (Figure 6C); again, no change was observed in control flies. These results demonstrate that activation of DM4 ORNs evokes attraction in our setup.

Dependence of Relative Valence on the Olfactory Context

One interpretation of the normalization model is that the attractive or aversive effect of a glomerulus is modulated by the

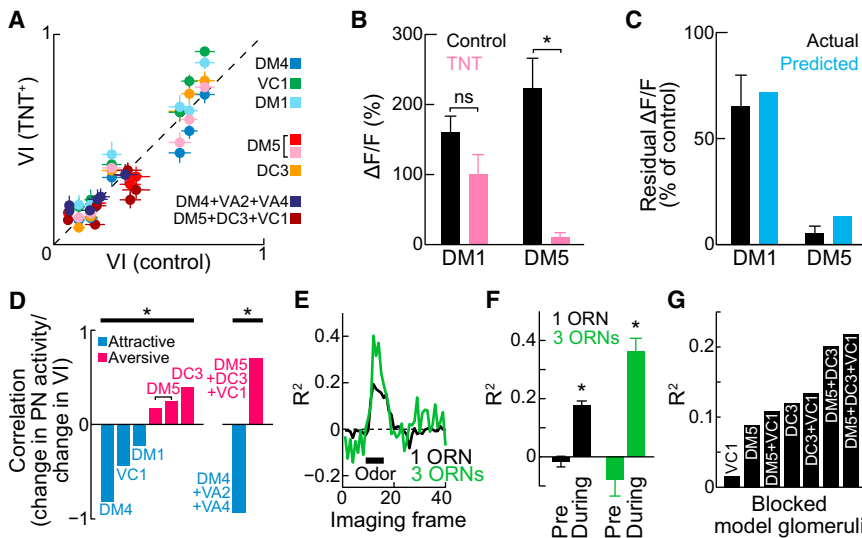


Figure 5. Behavioral Effect of Genetically Blocking Odor-Evoked Activity in Subsets of ORNs

(A) Comparison of VIs in TNT-positive (progeny of *Or-Gal4* × *UAS-TNT*) and control (*UAS-TNT/+*) flies. Identity of glomeruli in which ORN output is blocked is indicated in color legend. Error bars show SEM across flies. Each genotype ($n = 21$ – 26 flies) was tested using a set of six odors, except DM5, which was tested with two odor sets (12 stimuli).

(B) Comparison of Ca^{2+} responses of the PNs in glomerulus DM1 and DM5 to ethyl acetate with or without TNT expression in their cognate ORNs (mean $\pm$ SEM). TNT expression significantly reduced the response of DM5, but not DM1 PNs (two-sample t test, $p < 0.05$). DM5 response in TNT-expressing flies is not significantly different from zero (two-tailed t test, $p = 0.22$).

(C) Percentage of reduction in Ca^{2+} fluorescence due to TNT expression (data in B) compared with the predictions of a model in which ORN-PN synaptic transmission is blocked but PNs receive

excitatory input through lateral interactions between glomeruli. The prediction is computed from the Ca^{2+} responses recorded in GCaMP6f flies using an inferred PN-PN connection matrix (see Figure S5 and Supplemental Experimental Procedures).

(D) Pearson correlation between TNT-induced changes in PN activity and observed changes in behavioral response (six values for each condition; glomerulus DM5 tested with two different odor sets). Odor-evoked PN activity in TNT-positive flies is computed as in (C) and normalized according to the normalization model. The correlation of the pooled data was significant (two-tailed t test, $p < 0.01$), in both cases where either one (left) or three (right) ORN types were blocked. (E) R^2 of predicted change in VI for the case where one or three ORN types were blocked, shown as a function of imaging time frame. Odor application period is indicated by a black bar (4 s).

(F) Mean prediction performance for the case where either one or three ORN types were blocked. The R^2 averaged over three consecutive imaging frames is shown before odor application (Pre, frames 3–5), and during odor application (During, frames 12–14). Error bars indicate SD over the three imaging frames. Asterisks indicate statistical significance ($p < 0.006$, permutation test).

(G) Prediction performance as a function of the identity of blocked glomeruli in the model, showing additive effects of glomerular blockade.

olfactory context. From the point of view of a PN, an olfactory context is characterized by a specific history, or profile, of activation, with different contexts giving rise to different activation profiles. In a hypothetical example shown in Figure 7A, an aversive glomerulus responds similarly to a set of odors in context A, thus making its activation profile narrow (top red histogram). The normalization procedure broadens this histogram (bottom red histogram), enhancing differences between odor responses (responses to odor 1 and 2 become more separated). On the other hand, the difference between odor responses becomes suppressed in an attractive glomerulus having an initial broad profile (top blue histogram). When considering valence integration over two glomeruli, odor 1 in this example is more attractive than odor 2 ($VI_2 - VI_1 > 0$). Intriguingly, if we assume mirror symmetric activation profiles in context B, odor 1 would become more aversive than odor 2, even though the glomerular responses to the two odors (indicated by large arrowheads) are identical in both contexts (only the contextual activation by other odors indicated by small arrowheads, differ). In sum, the relative valence of two odors may vary depending on the identity of the other odors presented in the same experiment. In the extreme, the preference between two odors may switch when sampled in particularly different contexts (Figure 7A).

To test whether this phenomenon actually occurs, we measured behavioral responses to five different odor pairs, each of which was tested in two different olfactory contexts consisting of four other odors (Figure 7B). We computed the relative

valence as the difference in VI between the two odors in the pair and tested whether and how the relative valence depended on the olfactory context. We found that the relative valence was indeed affected by the other odors in the set (Figure 7C). The observed changes were relatively small; however, the predictions of our model consistently matched the data, and this effect was statistically significant ($p < 0.03$, permutation test, Figure 7C). Remarkably, the relative valence switched sign in several cases, demonstrating that perceptual decisions can be modulated by the olfactory environment.

The accuracy of model predictions was robust. On average, the direction of change in relative valence was more accurately predicted as the number of glomeruli in the model increased (Figure 7D, left), indicating that this effect also depends on summing small contributions over many glomeruli. Moreover, the accuracy of the predictions correlated well with the prediction performance of the model (Figure 7D, right), showing that models that predict the valence of individual test odors well also tend to perform better at predicting contextual changes in relative valence.

To gain further insight into this phenomenon, we tested the influence of abruptly changing the olfactory context during the experiment. Using the same odor sets, we presented the first context during the first half of the experiment (trials 1–8) and switched to the second context during the second half (trials 9–15). This should in principle inform us on the timescale of adaptation to the olfactory context. When we compared these

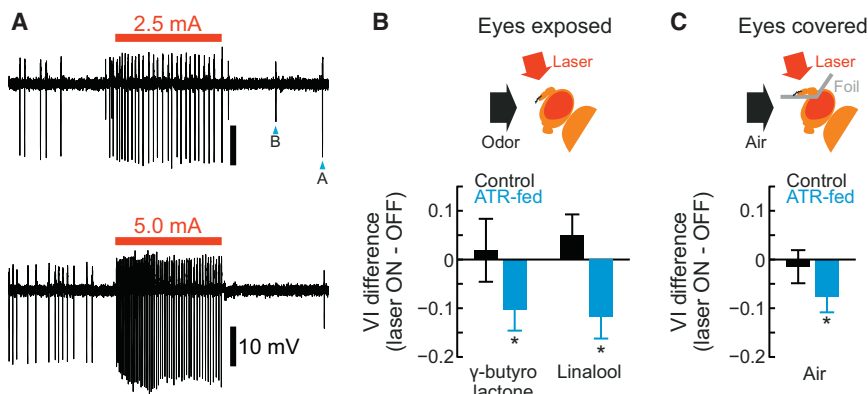


Figure 6. Optogenetic Activation of DM4 ORNs Evokes Attractive Behavior

(A) Responses of a Chrimson-expressing DM4 ORN (ab2A neuron) to a 637-nm laser light pulse at two different intensities. Red bar indicates period of laser application (1 s). Blue arrows denote the distinctive A and B spikes produced by the two neurons housed in the sensillum.

(B) Difference in VI between laser ON (5.0 mA) and laser OFF conditions in response to two odors, for Chrimson-positive ($n = 21$) and control ($n = 24$) flies (mean ± SEM). Both are of the same genotype, *Or59b-Gal4;UAS-Chrimson*, but control flies were not fed all-trans retinal (ATR). ATR-fed flies display significantly more attractive responses when odors are paired with laser (Wilcoxon signed-rank test, Bonferroni-corrected, $p < 0.05$).

(C) Difference in VI between laser ON (12.5 mA) and laser OFF conditions without odorant stimulus, for ATR-positive ($n = 17$) and control ($n = 21$) flies (mean ± SEM). Laser application was restricted to antennae by covering eyes and head cuticle with aluminum foil. ATR-positive flies show a significantly more attractive VI (Wilcoxon signed-rank test, $p < 0.05$).

data with the data obtained by sampling odor sets separately in different animals, we found that the results agreed: although changes in relative valence measured in the same animals tended to be smaller than those measured in different flies, the sign of the change was the same except for one case (Figure 7E). Interestingly, a model in which the relative valence of odors presented in the second half of the experiment is computed relative to a broader context, consisting of the sum of the first and second contexts, also predicts that the changes in relative valence should be smaller when the two contexts are presented sequentially (Figure S6). This suggests that in the second half of the experiment (10 to 20 min after the start of the experiment), flies are still affected by the odors sampled during the first 10 min, implying that adaptation occurs relatively slowly over a timescale of several minutes.

DISCUSSION

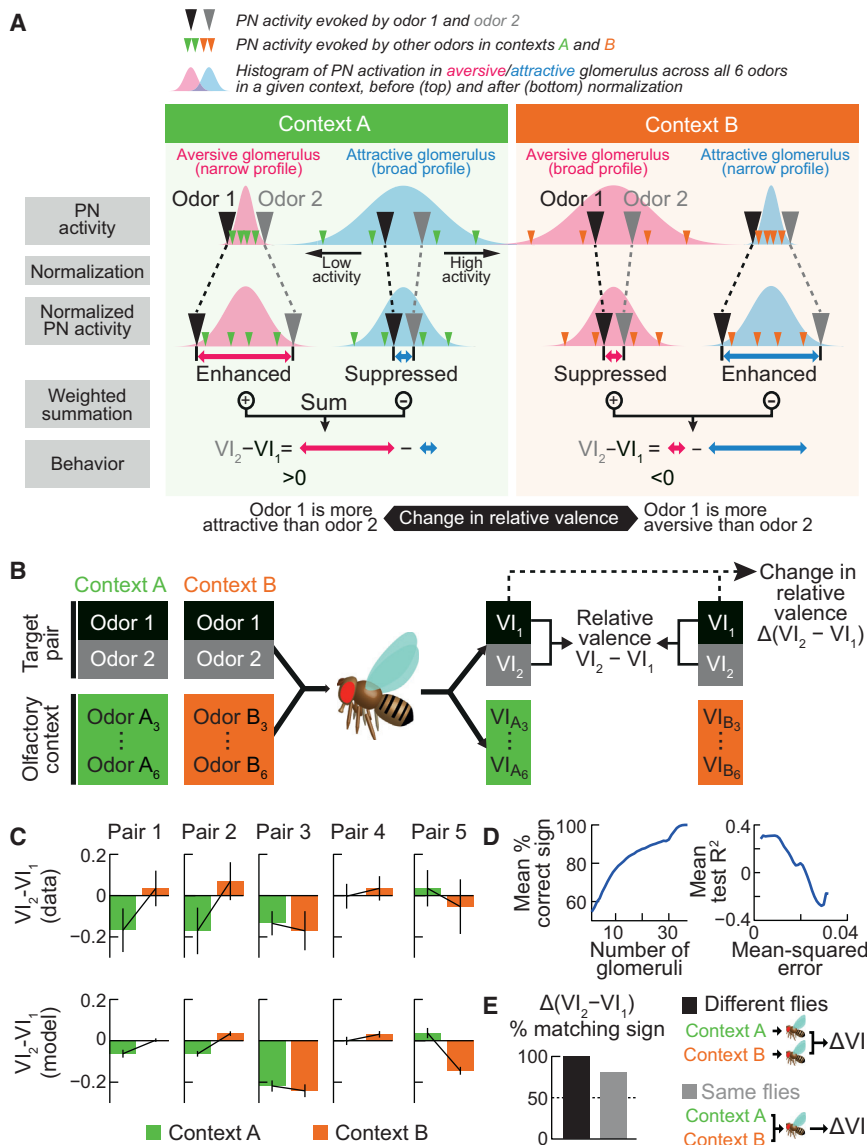
The aim of this study was to search for a general olfactory code—revealing the quantitative relationship between population neural activity and olfactory behavior that holds true for various odors under different contexts. Previous investigations have established how information about olfactory stimuli is encoded as patterns of glomerular activity in the olfactory bulb in vertebrates and the AL in insects. However, the computation that allows decoding of these activity patterns to guide behavior remained elusive. Here we present a model that can predict a fly's responses to novel odors and the way these responses adaptively change according to the olfactory environment.

A Decoding Model of Context-Dependent Olfactory Perception

There are several prerequisites for constructing a decoding model of perceptual performance. First, it is essential to obtain neuronal and behavioral data that are directly comparable. Most studies in *Drosophila* recorded transient neural activity in fixed preparations while scoring behavior integrated over an extended period of time in another setting (Knaden et al., 2012; Kreher et al., 2008; Parnas et al., 2013; Ronderos et al., 2014;

Semmelhack and Wang, 2009; Suh et al., 2004). Here, we made tethered individual flies navigate in a flight simulator so that identical, temporally aligned stimuli can be delivered during behavior and physiology experiments. A second requirement is to obtain physiological signals from a sufficiently large portion of neurons carrying the relevant sensory information. We took advantage of the *Drosophila* AL circuit, which is numerically and physically compact enough to be fully scanned by two-photon Ca^{2+} imaging, and whose outputs reach brain regions involved in memory and innate behaviors (Masse et al., 2009). Finally, it is necessary to cover a sufficiently large stimulus space, a requirement which is particularly stringent in olfaction where clearly defined axes for describing stimuli are lacking. We fulfilled these requirements by imaging PN dendritic activity in three quarters of the glomeruli at ~ 1.7 Hz, in response to 84 stimuli with diverse chemical structures, including binary mixtures and odorants at different concentrations. This comprehensive dataset (Table S2) could serve as a central counterpart of resources on odor representation by ORNs (Münch and Galizia, 2016).

Combining behavioral and physiological data allowed us to formulate a model explaining how behavioral responses can be decoded from glomerular activity. This simple model consists of a normalization step, operating independently on individual glomeruli, followed by a linear summation over all glomerular channels. Linear summation was naturally suggested by behavioral and physiological responses to binary mixtures and was sufficient to explain our data. Previous studies on mitral/tufted cells of the olfactory bulb and PNs of the insect AL reported results ranging from linear (Davison and Katz, 2007; Gupta et al., 2015; Khan et al., 2008) to non-linear interactions of the mixture components (Davison and Katz, 2007; Giraudet et al., 2002; Niessing and Friedrich, 2010; Shen et al., 2013; Tabor et al., 2004). However, because none of these studies examined behavior, it remained unknown whether glomerular output directly reflects innate olfactory perception. Here, we found that a linear model provided a good fit to the data. More importantly, behavioral responses mirrored the gradual change in PN activity. We do not claim, however, that non-linear interactions



the two contexts were sampled independently by different animals (same data as in C). Gray: "same flies" condition, in which the two contexts were presented sequentially to the same animals ($n = 40$ – 41 flies for each pair of odor sets). Dotted line indicates chance level (50%).

between glomeruli do not exist. For example, the efficacy of a glomerulus could be enhanced or depressed when it is co-activated with specific other channels. Nevertheless, given the accuracy of the linear framework, if such interactions do exist they are likely to be relatively weak or take effect in a stimulus range outside of our odor set.

A remarkable feature of our normalization model is its prediction that relative valence of odors can change depending on the olfactory environment. Behavioral experiments indeed showed that the fly's relative odor preference scales, and even switches, in many cases. Normalization is ubiquitous in the brain (Carandini and Heeger, 2011), and also in the AL, where it is reportedly implemented by short-term depression at the ORN-PN synapse (Kazama and Wilson, 2008) and divisive inhibition within the AL

Figure 7. The Relative Valence of Pairs of Odors Depends on the Olfactory Context

(A) Description of a hypothetical mechanism underlying context-dependent changes in relative valence in the normalization model. Different olfactory contexts are characterized by different profiles of PN activation, represented as Gaussians, determined by PN responses to the six odors in the context (arrowheads). According to the model, the attractive or aversive effect of a PN is enhanced if its activation profile in a given context is narrow, but weakened if it is broad (see text for details). As a consequence, the relative VI ($VI_2 - VI_1$), determined by summing differences in normalized PN activity over glomeruli, becomes context dependent.

(B) Schematic of experimental procedure. Two odor sets are prepared that include a pair of odors in common (odor 1 and 2). The remaining four odors define the olfactory context. Behavioral responses to both sets are measured independently, and the relative valence of the common odors is computed for each set as the difference in VIs ($VI_2 - VI_1$). The context-dependent change in relative valence is defined as the difference in this value between two contexts $\Delta(VI_2 - VI_1)$.

(C) Comparison of observed changes in relative valence with predictions of the normalization model, showing good agreement ($p < 0.03$, permutation test). Five odor pairs were obtained using four odor sets ($n = 21$ – 23 flies for each set). Pair 1: methanoic acid/1-octanol. Pair 2: methanoic acid/acetophenone. Pair 3: 1-octanol/acetophenone. Pair 4: 2-butanone/1-butanol. Pair 5: methanoic acid/1-butanol. Error bars show SEM (top, across tested flies; bottom, across 9 sets of glomerulus weights obtained in cross-validation).

(D) Left: average percentage of correctly predicted signs of change in relative valence, as a function of the number of glomeruli included in the model. Right: average prediction performance of the model as a function of the mean-square error on the prediction of changes in relative valence.

(E) Percentage of odor pairs for which the direction of change in relative valence was correctly predicted. Black: "different flies" condition, in which

network (Olsen et al., 2010). We modeled normalization using mean subtraction and division by the SD, two widely used operations to explain neuronal adaptation occurring at a short timescale (Baccus and Meister, 2002; Chander and Chichilnisky, 2001; Nagel and Doupe, 2006; Ohzawa et al., 1985). Here, we show that this framework can also explain behavioral adaptation occurring over a longer timescale.

It would be of interest to determine where and how the normalization operation described here takes place in the olfactory circuit. Mean subtraction is a form of high-pass filtering, which could be implemented through short-term synaptic plasticity (Abbott and Regehr, 2004), for example at synapses between PNs and third-order neurons. Alternatively, it may be supported by spike-frequency adaptation (Benda and Herz, 2003). For the

divisive component, it is not immediately obvious how a standard deviation could be computed; however, alternative models are possible. For example, in our data the SD correlated highly with the maximal activity, so that a model that rescales glomerular efficacy by the maximal activation performs equally well (data not shown). This simple form of gain control might be implementable, for example, by slow synaptic depression (Rothman et al., 2009). Although our data do not allow us to specify the exact mechanism of normalization, a nonlinear processing step is necessary to explain context-dependent changes in relative valence.

Rapid Odor Valuation by Tethered Flies

Earlier studies using tethered flies characterized how they modulate their flight upon encountering olfactory cues, but with few odors (Bhandawat et al., 2010; Frye and Dickinson, 2004; Guo and Guo, 2005; Wolf and Heisenberg, 1991). It therefore remained unexplored whether flies express fixed or graded responses depending on olfactory identity. We exposed flies to a large panel of odors and found a spectrum of responses. This suggests that flies are able to discriminate various odors and take graded actions. The difference in initial response was manifest within several hundreds of ms, demonstrating that flies can not only detect but also interpret odors rapidly. This initial decision correlated with the proportion of time spent outside the odor after prolonged exploration, suggesting that it reflects innate odor valuation. The short timescale of perceptual decision parallels that of the rodent's single sniff, which is sufficient to discriminate odors (Uchida and Mainen, 2003; Wesson et al., 2008). Given that odors in a natural environment are found in the form of plumes, which a navigating fly can cross in an instant (Murlis et al., 1992), the duration of each odor sampling may be comparable to, or even shorter than, a sniff. Therefore, rapid odor valuation is likely to be a fundamental requirement for efficient odor-guided navigation in complex environments.

Coding of Odor Valence by Individual Glomeruli

Previous research identified a number of glomeruli that appear dedicated to processing specific odorants such as pheromones, or broader classes of odors such as acids (reviewed in Li and Liberman, 2015). In all cases, silencing the glomerulus inhibited a behavioral response, suggesting that information about the valence of the tested stimuli is exclusively encoded in the activity of these glomeruli. However, because these studies generally used few odorants, and only a small fraction of glomeruli were tested, it is unclear how these results generalize to broader odor sets, and whether similar conclusions may hold for each of the ~50 glomeruli.

Our model attributes a specific weight to each glomerulus; the valence of a glomerulus thus corresponds to the sign of the associated weight. This model accurately fitted the data and predicted responses to novel odors. However, this does not entail that a causal relationship exists between glomerular activity and behavioral responses. We therefore tested causality by genetically blocking ORN input to targeted glomeruli, and by optically enhancing ORN input to a single glomerulus. The resulting changes in behavior were broadly consistent with the predic-

tions of our model. Furthermore, when we compared glomerular valences obtained in our analysis with published data in which a causal link was demonstrated, we found that both agreed in most cases. Attraction was previously linked to the activation of DM1 and VA2 (Sammelhack and Wang, 2009), VM1 (Min et al., 2013), DC3 (Ronderos et al., 2014), and DA1 and VA6 (Schlieff and Wilson, 2007). Conversely, DA2 (Stensmyr et al., 2012), DM5 (Sammelhack and Wang, 2009), and DM2 (Gao et al., 2015) were reported to mediate aversive responses. Our data differ from these results in only two cases, DC3 and VA6, both of which are of aversive valence in our model. It should be noted, however, that although DC3 was shown to subserve attraction to farnesol in Ronderos et al. (2014), another study (Knaden et al., 2012) reported DC3 to be activated almost exclusively by aversive odorants. Moreover, the study reporting VA6-mediated attraction to geranyl acetate (Schlieff and Wilson, 2007) also found that it mediated aversive responses to higher concentrations of the same odorant. Our results are thus in agreement with the published literature, supporting the validity of our model.

Integration of Valence Information over Multiple Glomerular Channels

Although a causal effect on behavior had been demonstrated for some glomeruli, it remained unclear whether animals make decisions based on the entire AL output, or rely on a smaller subset of specialized glomeruli. Four lines of evidence in our data support the hypothesis that behavioral responses result from integrating valence information over a large number of glomerular channels. First, the predictive power of the model increased as more glomeruli were added to the set of independent variables. Second, genetically blocking synaptic input to subsets of glomeruli had small and graded effects on behavior. This observation is in line with a previous study of odor discrimination (Parnas et al., 2013), in which silencing subsets of glomeruli evoked graded behavioral effects. Third, blocking input to every tested glomerulus biased behavior in a direction consistent with the model. Fourth, the extent of this behavioral bias was quantitatively predicted by our population decoding model.

Our model naturally accounts for findings on the processing of both specific and general odors: whereas behavioral responses to odors that exclusively activate narrowly tuned receptors can be explained solely by the activity of the corresponding glomeruli, responses to general odors recruiting multiple receptors need to be explained by an integrated output from the activated glomerular ensemble.

In *Drosophila*, innate olfactory behavior depends primarily on the lateral horn (LH), one of two brain regions postsynaptic to the AL (de Belle and Heisenberg, 1994; Heimbeck et al., 2001; Parnas et al., 2013). The LH is innervated by the excitatory PNs examined in the present study and by inhibitory PNs (iPNs; Liang et al., 2013; Parnas et al., 2013; Strutz et al., 2014; Wang et al., 2014). Whereas one study reported that a subset of iPNs responds differentially to attractive and aversive odors and contributes to behavioral expression (Strutz et al., 2014), another study (Parnas et al., 2013) found no effect of iPNs on innate responses, suggesting a major role of excitatory PNs in odor valuation. A better view on the interaction between these pathways

requires studying how the population code described here in the AL is transformed in the LH.

EXPERIMENTAL PROCEDURES

Fly Stocks

Flies were raised on conventional cornmeal agar medium under a 12 hr light/12 hr dark cycle at 25°C. Experiments were performed on 2- to 4-day-old adult females. Flies were starved for 4–6 hr with water prior to experiments. Flies used in the study are listed in Table S3.

Olfactory Stimulation

Odors were delivered by passing an air stream through 4 mL of odorant solution in a vial. A portion of the air stream was delivered through a ϕ 2 mm outlet placed 10 mm from the fly. Air flow was set to 0.3 m/s at fly location. A total of 84 odors were used (Table S1).

Behavioral Experiments

Flies were cold anesthetized, tethered to a stainless steel pin, and transferred to the flight simulator, which consisted of an odor delivery apparatus and a semi-circular array of green LEDs. The setup was enclosed in an opaque container and the fly was visualized using an infrared camera.

Flight behavior was monitored using two microphones positioned laterally $\sim$ 1 mm from the tip of the extended wing on either side of the fly, whose outputs were analyzed in real time to extract turning direction and speed. The validity of computing turning propensity from microphone data was verified by comparing with video data (Figure S1). The visual stimulus (essential to increase flight reliability) consisted of vertical gratings with 60 deg^{−1} spatial frequency. The panel of 84 odors was divided in sets of 6 (Table S1) and a single set was applied in each experiment (except Figure 7E, “same flies” condition, where two sets were applied consecutively). The protocol consisted of 15 blocks, in which each of the 6 odors plus mineral oil were applied in randomized order, up to a fixed duration (4 s unless otherwise stated) and in a restricted spatial region (45° centered at the fly location at the time of odor contact). Odor application was terminated when the fly exited this region and re-initiated upon re-entry within the application period. For optogenetic activation experiments, a 637 nm laser targeted at the antennae was controlled in closed-loop similarly as odorant stimuli.

Two-Photon Imaging

Individual flies were attached to a custom recording plate with ultraviolet-curing adhesive while cold-anesthetized. Saline bubbled with 95% O₂/5% CO₂ was added and the head cuticle was removed to expose the brain.

The entire right AL was imaged with a two-photon laser scanning microscope. The fluorophore was excited with a titanium:sapphire pulsed laser mode locked at 930 nm. Each odor was presented for 4 s with 1 min inter-trial interval. Odor valve opened in frame 9 and closed in frame 16. Each odor was presented four times in randomized order as in behavioral experiments. After data collection, a high-resolution 3D image of the AL comprising 99 optical slices (1 μ m interval) was acquired for offline identification of guide-post glomeruli (see Data Analysis section).

Data Analysis

Behavioral Data Analysis

Valence indices (VIs) for individual flies were obtained by aligning flight trajectories to the time of odor contact (Figure S7) and calculating the proportion of time spent outside the plume. Instantaneous VIs were calculated in every 5 ms time bin, and the mean VI (simply referred to as VI throughout the text) was calculated as an average over the last 1 s of odor presentation. Mean and standard error were computed by pooling all tested flies. Δ WBA in Figure 1G was calculated using the absolute difference in the standardized wing-beat amplitudes (Supplemental Experimental Procedures). Trials in which flight was interrupted were excluded.

Two-Photon Imaging Data Analysis

Images were analyzed in three steps: correction for brain motion, creation of template glomeruli, and extraction of fluorescence changes in each glomer-

ulus using the template (Figure S3). Table S2 summarizes the mean response of each of the 37 glomeruli to the 84 odors in our dataset.

Decoding Analysis

For the normalization model, PN data were standardized in each glomerulus using mean and standard deviation values computed independently for each set of six odors, by pooling trial-averaged $\Delta F/F$ responses (imaging frames 12 to 16) over all six odors. VI values were similarly centered by subtracting their mean value over the six odors. The model was fit using partial least-squares regression and a cross-validation procedure to control for overfitting. Prediction performance was assessed using the R² coefficient and statistical significance using a permutation test.

See Supplemental Experimental Procedures for detailed methods.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, three tables, and two movies and can be found with this article online at <http://dx.doi.org/10.1016/j.neuron.2016.05.022>.

AUTHOR CONTRIBUTIONS

L.B. and H.K. conceived and designed the study. L.B. and H.K. constructed the flight simulator with input from Hiroshi M. Shiozaki, and the olfactometer with input from Keita Endo. L.B. and K.O. performed behavioral experiments and analyzed data. Y.T. and H.K. performed imaging experiments and analyzed data. L.B. performed single sensillum recordings. L.B. and H.K. performed decoding analyses and wrote the manuscript.

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