**Divisive Normalization in Olfactory Population Codes**

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**SUMMARY**

In many regions of the visual system, the activity of a neuron is normalized by the activity of other neurons in the same region. Here we show that a similar normalization occurs during olfactory processing in the *Drosophila* antennal lobe. We exploit the orderly anatomy of this circuit to independently manipulate feedforward and lateral input to second-order projection neurons (PNs). Lateral inhibition increases the level of feedforward input needed to drive PNs to saturation, and this normalization scales with the total activity of the olfactory receptor neuron (ORN) population. Increasing total ORN activity also makes PN responses more transient. Strikingly, a model with just two variables (feedforward and total ORN activity) accurately predicts PN odor responses. Finally, we show that discrimination by a linear decoder is facilitated by two complementary transformations: the saturating transformation intrinsic to each processing channel boosts weak signals, while normalization helps equalize responses to different stimuli.

**INTRODUCTION**

Sensory neurons are selective for specific stimulus features. For example, a neuron in primary visual cortex may be sensitive to both the spatial location and the orientation of a stimulus. Similarly, the preferred stimulus of an olfactory neuron is defined by the molecular features of the odors that are effective at driving that neuron. Stimuli with nonpreferred features often have an inhibitory effect on a sensory neuron. The earliest illustrations of this principle came from studies of neurons in the *Limulus* eye (Hartline et al., 1952) and vertebrate retina (Barlow, 1953; Kuffler, 1953). These neurons respond best to light at a particular spatial location, and responses to light at the best position can be suppressed by simultaneously illuminating other locations. This concept was later extended to features other than spatial location. For example, it was observed that in primary visual cortex, a neuron’s response to a grating with a preferred orientation can be suppressed by superimposing a nonpreferred orientation (Morrone et al., 1982).

The idea linking these findings is that a neuron’s response to a preferred stimulus feature is inhibited by adding nonpreferred stimulus features. This phenomenon can be understood as a form of “gain control,” defined as a negative feedback loop that keeps the output of a system within a given range. It has been proposed that this type of gain control in the visual system works by performing a divisive normalization of neural activity (Heeger, 1992). According to the divisive normalization model, the response of a neuron to a complex stimulus is not the sum of its responses to each stimulus feature alone. Rather, the response is divided by a factor related to the total “stimulus energy,” which increases with stimulus intensity and complexity. For this reason, the response of a neuron to a complex stimulus is closer to an average of its responses to each feature.

A fundamental question is how gain control alters the response of a neuron to its preferred stimuli. A neuron’s response to preferred stimuli is generally nonlinear, with intense preferred stimuli driving the neuron to saturation. It is important to define whether gain control scales the input to this function (thus making it more difficult to reach saturation) or the output of this function (diminishing the strength of the saturated response). Both forms of gain control seem to occur in visual processing and attentional control (Albrecht and Geisler, 1991; Cavanaugh et al., 2002; Williford and Maunsell, 2006; Reynolds and Heeger, 2009). Another important question is what cellular and circuit mechanisms form the substrate of this process. At least in some classic examples of gain control in visual processing, there is a clear role for lateral inhibition (Kuffler, 1953; Hartline et al., 1956).

One reason why these questions have been difficult to resolve is the complexity of the underlying circuits. Ideally, one would like to selectively manipulate feedforward excitation and lateral inhibition to the neuron one is recording from. From this perspective, the *Drosophila* antennal lobe is a useful preparation because of its compartmental organization (Figure 1A). All the olfactory receptor neurons (ORNs) that express the same odorant receptor project to the same glomerulus in the brain, where they make excitatory synapses with projection neurons (PNs). Each PN receives ORN input from one glomerulus and lateral inputs from other glomeruli (Bargmann, 2006). A PN’s odor responses are disinhibited by silencing input to other glomeruli (Olsen and Wilson, 2008; Asahina et al., 2009), implying that lateral interactions are mainly inhibitory. This could explain the observation that a PN’s response to an odor can be inhibited by adding a second odor that is ineffective at driving that PN when presented alone (Deisig et al., 2006; Silbering and Galizia, 2009).
orn (defined as stimuli that selectively activate a population of other stimuli with varying concentrations of “public” stimuli). The aims of this study are to understand how lateral inhibition alters the response of a PN to its presynaptic ORNs and how this type of gain control affects PN population codes for odors. Previous studies have used odor stimuli that activate multiple ORN types, thereby driving both direct and lateral input to a PN. Instead, here we begin with “private” stimuli, defined as stimuli that activate only one ORN type (Figure 1A). By mixing private stimuli with varying concentrations of “public” stimuli (defined as stimuli that selectively activate a population of other glomeruli), we measure how increasing activity in other glomeruli suppresses the response of a PN to its presynaptic ORNs.

RESULTS

A Uniform Intraglomerular Transformation

Based on a previous study (Hallem and Carlson, 2006), we identified four likely private odors and their cognate ORN types (Table S1). We sampled randomly from many ORNs of other types in order to confirm that these odors do not activate non-cognate ORNs (Figure S1). Moreover, where mutations were available in the cognate odorant receptors for these odors, we verified that they virtually abolish the response of the ORN population (Figure S1).

For each of the four associated glomeruli, we recorded the responses of both ORNs and PNs to a range of concentrations of their private odor. Responses were quantified as spike rates over the 500 ms stimulus period. We found that the input-output relationships for three of these glomeruli were very similar (Figure 1B). In all these cases, weak ORN inputs were selectively boosted and strong inputs saturated. In the fourth glomerulus, the relationship between PN and ORN responses was shallower, but when GABA receptor antagonists were added, this relationship reverted to the typical steeper shape. The antagonists had no effect on a more typical glomerulus (Figure 1B).

These results suggest that all glomeruli perform a similar transformation on their inputs, although in some cases this transformation is modified by GABAergic inhibition. We can formalize this by fitting all these input-output relationships with the same equation:

$$PN = R_{max} \left( \frac{ORN^{1.5}}{ORN^{1.5} + \sigma^{1.5}} \right)$$

where $PN$ is the response of an individual PN to a private odor stimulus, and $ORN$ is the response of an individual presynaptic ORN to the same stimulus. $R_{max}$ is a fitted constant representing the maximum odor-evoked response, and $\sigma$ is a fitted constant representing the level of ORN input that drives a half-maximum response. $R_{max}$ and $\sigma$ are essentially the same for all glomeruli ($10^{-10}$, antagonists $\sigma$ is larger for the fourth glomerulus we examined). The saturating form of this function reflects the combined effects of short-term depression at ORN-PN synapses and the relative refractory period of PNs (Kazama and Wilson, 2008). In Equation 1, the input terms are raised to an exponent of 1.5 because this produced the best fit; a similar equation describes the contrast response functions of visual neurons, and there too an exponent $>1$ is generally required (Albrecht and Hamilton, 1982; Heeger, 1992; Reynolds and Heeger, 2009; see Discussion).

Lateral Interactions Are Inhibitory

We next asked how activity in other glomeruli affects a PN’s response to its cognate ORNs. Here we focused on two glomeruli: VM7 and DL5. In order to manipulate input to other glomeruli independently from input to these glomeruli, we used a “public” odor that activates many ORN types but not these ORNs (Figure 1A). We verified that this odor (pentyl acetate)
does not activate either VM7 or DL5 ORNs (at dilutions up to $10^{-3}$, see Figure S2). Thus, varying the concentration of pentyl acetate allows us to vary total ORN activity, as measured by field potential recordings in the antenna (Figure 2A).

We mixed pentyl acetate with 2-butanone (the private odor for VM7 ORNs) at various concentrations, generating 20 stimuli in total that we then tested on VM7 PNs. We found that pentyl acetate inhibited the responses of VM7 PNs to 2-butanone, with higher concentrations producing more inhibition (Figures 2B and 2C). The effect of pentyl acetate was blocked by GABA receptor antagonists (Figure 2D), as expected.

Similar results were obtained for a second glomerulus: DL5. Here we mixed pentyl acetate with trans-2-hexenal, the private odor for DL5 ORNs (Figure 2E). The magnitude of inhibition was consistently smaller for DL5 than for VM7, implying that glomeruli differ in their sensitivity to lateral inhibition.

**Lateral Inhibition Normalizes Input**

We next asked whether lateral inhibition scales the horizontal or vertical axis of the input-output function (Figures 3A and 3B). We term horizontal scaling “input gain control.” We can express this by adding a suppression factor $s$ to the denominator of the hyperbolic ratio function:

$$PN = R_{\text{max}} \left( \frac{\text{ORN}_{15}}{\text{ORN}_{15} + s_{15} + \sigma_{15}} \right) \quad (2)$$

We term vertical scaling “response gain control” (Figure 3B), and we can express this by scaling $R_{\text{max}}$.

$$PN = \left( \frac{1}{s_{15} + 1} \right) R_{\text{max}} \left( \frac{\text{ORN}_{15}}{\text{ORN}_{15} + \sigma_{15}} \right) \quad (3)$$

We fit both these models to the data in Figure 2, fixing $R_{\text{max}}$ and $\sigma$ at the values we obtained from the curves in Figure 1B and letting $s$ be a fitted variable that varies with the concentration of pentyl acetate.

We found that for both VM7 and DL5, the input gain model generated better fits than the response gain model (Figures 3C–3G). This reflects the fact that responses to dilute private odor were suppressed more powerfully in proportional terms than responses to concentrated private odor. The input gain model was also better than two subtractive models (see Supplemental Experimental Procedures). Thus, the effects of lateral inhibition are best described as input gain control.

**Lateral Inhibition Scales with Total ORN Activity**

How does the level of inhibition in a given glomerulus depend on the pattern of activity in the ORN population? It is possible that each glomerulus might receive strong inhibitory input from just a few glomeruli. However, many individual GABAergic local neurons in the antennal lobe innervate most glomeruli (Das et al., 2008; Lai et al., 2008), suggesting that they pool excitation from most ORN types and inhibit each glomerulus by a factor that depends on the total activity of this ORN population. If this were true, then our data should reveal a clear relationship between $s$ and total ORN activity, assuming all glomeruli contribute equally to the pool.
To test this prediction, we asked how $s$ depends on total ORN activity. We obtained $s$ using Equation 2 for each concentration of pentyl acetate, again with $R_{\text{max}}$ and $\alpha$ held constant at the values obtained from the curves in Figure 1B. For each concentration of pentyl acetate, we obtained an estimate of total ORN activity by measuring the antennal local field potential (LFP; Figure 2A) because this scales linearly with ORN activity (Figure S3). We found that the relationship between $s$ and LFP was linear for both VM7 and DL5 (Figure 3H). Thus, 

$$s = m \cdot \text{LFP}$$

where the slope $m$ represents the sensitivity of each glomerulus to lateral inhibition. (Note that $m$ is larger for VM7 than for DL5; Figure 3H.) The linear relationship between $s$ and LFP implies these glomeruli are normalized by an amount that simply scales with total ORN activity.

If lateral inhibition in each glomerulus scales with total ORN activity, then the contribution of any single glomerulus to the inhibitory signal should be weak. We therefore asked whether stimulating one glomerulus can produce substantial lateral inhibition. We used private odors to drive robust activity (~100 spikes/s) in a single ORN type but not in VM7 ORNs. The ORN types activated by these odors were DM4, DL5, and DM1, and the three private stimuli were the highest concentrations of their cognate private odors in Figure 1. Mixing each private odor with 2-butanone produced only weak suppression of the VM7 PN response to 2-butanone (data not shown). This result is consistent with a model whereby interglomerular inhibitory connections are weak, and thus input to multiple glomeruli is required to evoke measurable lateral inhibition.

**Predicting PN Responses to Novel Odors**

These findings imply that we should be able to predict the odor-evoked firing rate of these PNs based on only two variables: the firing rate of their presynaptic ORNs and the firing rate of the total ORN population. To examine the quality of these predictions, we measured the responses of VM7 ORNs to a set of test odors that were not used to construct our model. As a proxy for total ORN activity, we measured the antennal LFP for each test odor (Figures 4A and 4B). Next, we used these measurements to predict the odor responses of VM7 PNs on the basis of Equations 2 and 4, using the value of $m$ that represents the sensitivity of VM7 to lateral inhibition. Strikingly, predicted and measured PN firing rates were in excellent agreement, with the input gain model accounting for 95% of the variance in the data (Figure 4C). We repeated this procedure for glomerulus DL5, here using the value of $m$ derived for DL5. Again, the input gain model made very good predictions, accounting for 87% of the variance in the data (Figure 4D). The success of these predictions provides further support for the conclusion that the suppression factor $s$ varies linearly with the LFP (Figure S4). As expected, the response gain control model did not accurately predict PN responses (data not shown).

**Gain Control Reformats Population Codes**

What are the consequences of these transformations for the way odors are encoded at the population level? To address this, we first examined the statistical properties of ORN population codes. We then used our model to simulate PN population codes and ask how their properties are altered as compared to ORNs. Ultimately, we are interested in how these transformations affect odor discrimination.
As the input to our model, we used ORN odor responses measured by Hallem and Carlson (2006), comprising 176 olfactory stimuli and 24 ORN types. This data set displays a strong statistical regularity: stimuli that evoke a robust response in a given ORN type also tend to evoke robust responses in many ORN types (Figure 5A1). This can be quantified by principal components analysis on the odor response vectors, which shows that the first principal component (PC) accounts for fully 49% of the variance in the data. This PC is essentially a proxy for stimulus intensity (Figure S5). Another way to quantify this is to perform pairwise comparisons between ORN types, which shows that pairwise correlations are high (Figure 5B1). These correlations have an important corollary: because some stimuli elicit weak responses in many ORN types and others elicit robust responses, stimuli produce widely varying levels of total activity. We quantified this by computing the magnitude of the population response evoked by each stimulus, defined as the norm of the population response vector. This distribution is broad (Figure 5C1), meaning that total odor-evoked activity varies over a wide range. In short, all these analyses show that the responses of ORNs are not statistically independent.

To model PNs without inhibition, we simulated the intraglomerular transformation by applying Equation 1 to the ORN matrix. This transformation boosts the smallest responses, while pushing the largest responses toward saturation (Figure 5A2). This transformation does not reduce statistical dependencies between glomeruli: the first principal component still accounts for a high percentage of the variance in the data (52%). Consistent with this, pairwise correlations among glomeruli are largely unchanged (Figure 5B2). This is because some stimuli still recruit strong responses across the population whereas other stimuli do not, and this means that the distribution of population response magnitudes remains broad (Figure 5C2).

Next, we added lateral inhibition using the input gain control model. This requires us to know the total level of ORN activity evoked by each odor. Instead of taking LFP measurements for all these odors, we obtained an expression for $s$ as a function of ORN firing rates. We measured LFP responses to a subset of the stimuli in the ORN data set, and we fit a line to the relationship between these LFP responses and the total number of ORN spikes evoked by each odor (Figure S3). The fitted line is given by:

$$LFP = \left( \sum_{i=1}^{24} r_i \right) / 190 \text{ mV} \cdot \text{s}^2 / \text{spikes}$$

where $r_i$ is the firing rate of the $i$th ORN type. Combining Equations (5) and (4) we obtain:

$$s = m \cdot \left( \sum_{i=1}^{24} r_i \right) / 190 \text{ mV} \cdot \text{s}^2 / \text{spikes}$$

The constant $m$ in Equation 6 was obtained from the fit to VM7 data with the input gain model (Figure 3H). By combining Equations 2 and 6, we were able to simulate the ORN-PN transformation according to the input gain model (Figure 5A3). This transformation counteracts the tendency for intense stimuli to recruit strong responses across the PN population, and for this reason it decorrelates glomeruli (Figure 5B3). It also decreases the magnitudes of the strongest population responses while leaving the weaker responses relatively unaffected, and as a result population response magnitudes are now more equal (Figure 5C3). As a result, the first principal component accounts for s as a function of ORN firing rates. We measured LFP responses to a subset of the stimuli in the ORN data set, and we fit a line to the relationship between these LFP responses and the total number of ORN spikes evoked by each odor (Figure S3). The fitted line is given by:
for a smaller percentage of the variance in the data (25% versus 52%).

To simulate response gain control (Figure 5A4), we combined Equations 3 and 6, and we obtained the constant m in Equation 6 by fitting this equation to VM7 data (data not shown). Like input gain control, this transformation decorrelates glomeruli (Figure 5B4) and decreases the variance accounted for by the first principal component (to 28%). Again, like input gain control, it also tends to equalize population response magnitudes (Figure 5C4). But whereas input gain makes it more difficult for PN responses to saturate, response gain control does nothing to prevent saturation. This means that intense stimuli evoke similar weak levels of activity in many PN types.

Finally, as a control, we shuffled the odor labels on each ORN response vector before computing s. In this case, inhibition does not decorrelate glomeruli or equalize population response magnitudes (data not shown). The key point is that gain control only produces decorrelation and equalization of responses if inhibition grows with increasing input to the circuit.

We simulated a set of 176 binary linear classifiers (perceptrons), one for each stimulus. The input to each perceptron was a weighted sum of all glomerular responses, and the perceptron responded if the sum exceeds its threshold. Input weights were constrained to be nonnegative, but they were adjusted for each perceptron so that it responded as selectively as possible to one stimulus. For each of the four response matrices (Figure 5A), we created a set of perceptrons with weights appropriate to that matrix. Training and test stimuli were created by adding noise to each response matrix, where the parameters of the noise were drawn from PN data (Figure S6). Each set of perceptrons was evaluated on the basis of its ability to correctly classify these noisy test stimuli. Thresholds were adjusted so that the fraction of false positives equaled false negatives.

First, we examined the case where PN responses are identical to ORN responses (i.e., no transformation, using the matrix in Figure 5A1). Perceptrons trained and tested on these responses performed relatively poorly (Figure 6A). Specifically, strong stimuli generated a high rate of false positives. This is because strong stimuli generate strong responses in many glomeruli,
and thus tend to drive the weighted sum in all perceptrons above threshold.

Next, we examined the effect of the intraglomerular transformation, without lateral inhibition. Perceptrons trained and tested on this matrix performed better (Figure 6B). This is because the intraglomerular transformation selectively boosts PN responses to weak ORN inputs. This makes it easier to find weights that yield a selective response to weak stimuli. However, every perceptron still tended to respond inappropriately to many strong off-target stimuli.

Input gain control largely solves this problem (Figure 6C). This is because this model normalizes PN responses by the total level of ORN input, and so strong stimuli no longer elicit so many false positives. By comparison, the response gain control model performs more poorly (Figure 6D). Like input gain control, this model has the virtue of normalizing responses to strong stimuli. However, this model compresses the PN dynamic range when the total level of ORN input is strong, and so strong stimuli elicit weak responses in all glomeruli. This makes it difficult to find a threshold that maximizes correct hits while also minimizing false positives.

Input Gain Control Promotes Intensity Invariance

Next, we asked perceptrons to respond selectively to an odor across a range of concentrations. This task is inspired by the experimental finding that some KCs respond selectively to a particular odor regardless of its concentration (Stopfer et al., 2003; Wang et al., 2004). Because we had available data on 19 odors at each of three concentrations, we trained 19 perceptrons on this task, one for each odor.

Again, we first examined the case where PN responses are identical to ORN responses (no transformation). These perceptrons did relatively poorly (Figure 7A) because low concentrations evoke such weak responses that they are not easily classified with high concentrations. The intraglomerular transformation improves performance (Figure 7B) because it selectively boosts weak responses, and so brings low and high concentrations closer together. Input gain control creates the best performance (Figure 7C) because it normalizes for intensity, and this makes responses to different concentrations more similar. Response gain control also normalizes for intensity, but it performs more poorly than input gain control (Figure 7D). Because high concentrations elicit intense lateral inhibition which suppresses all PN responses uniformly, these strong stimuli elicit small population responses, and it becomes difficult to maximize correct hits while minimizing false positives.

Increasing Total Activity Makes Responses More Transient

For simplicity, we have thus far quantified neural activity as mean firing rates over the stimulus period. However, PN responses do not remain constant over the stimulus period. In order to investigate how lateral inhibition shapes these dynamics, we compared...
the time course of PN responses to different levels of private and public input. We found that, as a general rule, mixing in a public odor tended to make PN responses to private input more transient (Figures 8A and 8B).

We quantified transience as the ratio of the peak firing rate to the mean firing rate (Figure 8C). As the public odor concentration increased, the peak-to-mean ratio systematically increased. This is probably because a strong public stimulus recruits ORNs faster. Consistent with this idea, higher public odor concentrations produce a faster increase in the antennal LFP (Figure 8D). Faster recruitment of the ORN population should recruit faster lateral inhibition, and thus more transient PN responses.

However, the effect of the public odor on PN dynamics was only large when the private odor concentration was low (Figures 8A–8C). This suggests that increasing total ORN activity only makes PN responses more transient when direct input is weak. This would be consistent with an input gain control model, because in this model the effect of lateral inhibition is strongest when PNs are far from saturation. Thus, input gain can account for why lateral inhibition affects the dynamics of some PN responses more than others.

**DISCUSSION**

**Normalization Models in Olfaction and Vision**

As originally formulated in the visual system, the normalization model of gain control includes two conceptually separate steps:

1. **Saturating Function**:
   
   $R(c) = \frac{R_{\text{max}}}{c^n + s^n}$

   where the variable $c$ is the contrast of the visual stimulus, $s$ is a constant, and $n$ is a constant exponent (generally empirically determined to be $> 1$).

2. **Normalization**: As the public odor concentration increased, the peak-to-mean ratio systematically increased. This is probably because a strong public stimulus recruits ORNs faster. As the public odor concentration increased, the peak-to-mean ratio systematically increased. This is probably because a strong public stimulus recruits ORNs faster. As the public odor concentration increased, the peak-to-mean ratio systematically increased. This is probably because a strong public stimulus recruits ORNs faster.

   The second step—normalization—has been modeled in the visual system as an increase in the contrast needed to drive a neuron to half-maximum firing rate:

   $R(c) = \frac{R_{\text{max}}}{c^n + s^n + q^n}$

   where the suppression factor $s$ depends on stimulus contrast and can be rather nonselective for other stimulus features, presumably reflecting summed input from neurons with diverse stimulus preferences (Heeger, 1992). Versions of this model describe neural activity in several visual cortical areas (Carandini et al., 1997; Cavanaugh et al., 2002; Zoccolan et al., 2005), and this model has also been extended to describe the effects of attention (Lee and Maunsell, 2009; Reynolds and Heeger, 2009). There are differences between the models in these studies; for example, $s$ can be either nonselective or selective. Generally an exponent $> 1$ is required to fit the data (Albrecht and Hamilton, 1982; Heeger, 1992; Carandini and Heeger, 1994; Reynolds and Heeger, 2009), although the mechanisms underlying this are uncertain. Nevertheless, the essential concept captured by this equation is simple: the activity of each neuron is normalized by activity in a larger pool of neurons.

   Here we show that a similar function (Equation 2) describes gain control in the *Drosophila* antennal lobe. By independently manipulating direct and lateral input to a PN, we show that the saturating transformation is intrinsic to each glomerular channel, whereas the normalization step is due to lateral inhibition. Thus,
at least in this circuit, these two transformations are not just conceptually distinct but also mechanistically distinct.

**Population Codes for Odors**

Our results show that both the intra- and interglomerular transformations promote odor discrimination by a linear decoder. First, the intraglomerular transformation selectively boosts weak ORN inputs. Because responses to weak stimuli are preferentially amplified, it becomes easier to find a combination of glomerular weights that produces a selective response to one of these stimuli. A recent theoretical study pointed out that this type of transformation should promote linear separation (Luo et al., 2008), and our results reinforce that conclusion. Second, the normalization step decreases the steepness of the intraglomerular transformation by a factor proportional to total input. As a result, activity in different glomeruli is decorrelated. This agrees with theoretical studies showing that normalization makes the responses of different neurons more statistically independent (Schwartz and Simoncelli, 2001). Another precedent for our results is a recent theoretical study pointing out that global lateral inhibition should decorrelate the odor selectivity of different glomeruli (Cleland and Sethupathy, 2006), although that study postulated a different type of intraglomerular transformation than the function we describe here. Importantly, we show that this type of normalization makes it easier for a linear decoder to respond selectively to a particular stimulus. This is because stimuli of different intensities now evoke population responses with a more similar magnitude.

It is useful to consider both of these steps—boosting and normalization—in terms of efficient coding. The efficient coding hypothesis has two parts: (1) each neuron should use its dynamic range uniformly, and (2) responses of different neurons should be independent (Simoncelli, 2003). Most ORN responses are weak, so ORNs do not use their dynamic range uniformly. By selectively boosting weak inputs, the intraglomerular transformation creates PN responses that use the available dynamic range more uniformly (Bhandawat et al., 2007). Meanwhile, most ORNs are also correlated with each other. By creating competitive interactions between neurons in different glomeruli, normalization decorrelates their responses. (Note the distinction between decorrelating neurons and decorrelating representations: global lateral inhibition does the former but not the latter; see Figure S7.)

A previous study reported that PN responses are not substantially more decorrelated than ORN responses (Bhandawat et al., 2007). Two considerations reconcile our findings with that study. First, we show here that although lateral inhibition tends to decorrelate PN odor responses, the intraglomerular transformation tends to correlate them. Thus, the net effect of both transformations is less decorrelating than lateral inhibition alone. Second, the previous study used stimuli spanning a narrow range of intensities. By contrast, the stimuli in our simulations here span a wide range, which leads to a larger decorrelation.

**Toward Concentration-Invariant Odor Representations**

Functional imaging studies in the olfactory bulb have shown that different concentrations of the same odor elicit different levels of activity in the bulb, but these spatial maps are similar after signals are normalized to the same amplitude (Johnson and Leon, 2000; Wachowiak et al., 2002; Cleland et al., 2007). For this reason, normalization via lateral inhibition has been proposed as a basis for concentration-invariant odor representations. It is useful to consider both of these steps—boosting and normalization—in terms of efficient coding. The efficient coding hypothesis has two parts: (1) each neuron should use its dynamic range uniformly, and (2) responses of different neurons should be independent (Simoncelli, 2003). Most ORN responses are weak, so ORNs do not use their dynamic range uniformly. By selectively boosting weak inputs, the intraglomerular transformation creates PN responses that use the available dynamic range more uniformly (Bhandawat et al., 2007). Meanwhile, most ORNs are also correlated with each other. By creating competitive interactions between neurons in different glomeruli, normalization decorrelates their responses. (Note the distinction between decorrelating neurons and decorrelating representations: global lateral inhibition does the former but not the latter; see Figure S7.)

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representation in the olfactory bulb (Johnson and Leon, 2000; Wachowiak et al., 2002; Cleland et al., 2007) and the antennal lobe (Sachse and Galizia, 2003; Asahina et al., 2009). Here, we provide evidence for this computation at the level of ORN input to Drosophila antennal lobe glomeruli.

*Glomerulus-Specific Sensitivity to Inhibition*

Our results show that glomeruli differ in their sensitivity to lateral inhibition. This appears as differing values of the factor $m$ that expresses how steeply lateral inhibition depends on total ORN activity. Although we examined only two glomeruli in detail, our analysis of a published data set comprising seven additional glomeruli (Bhandawat et al., 2007) suggests that the values of $m$ for VM7 and DL5 fall within the typical range. Another finding from this study is that one of the four glomeruli we examined (DM1) is modulated by inhibition arising from odor-evoked intraglomerular GABA release and/or tonic interglomerular GABA release. This appears as a higher value of the semisaturation constant $\alpha$ for this glomerulus.

This heterogeneity does not affect our overall conclusions about the consequences of gain control. If instead of using the value of $m$ for VM7 as the default we randomly assign to each glomerulus a value of $m$ intermediate between the values for VM7 and DL5, then the overall effects of inhibition are weaker but qualitatively unchanged. Similarly, the results of our simulations are qualitatively unchanged if we randomly assign a high value of $\alpha$ to a subset of glomeruli (data not shown).

Given this, it is worth asking why heterogeneity might be useful. We speculate that some glomeruli might be specialized in their sensitivity to GABAergic inhibition because they respond preferentially to an odor with special behavioral relevance or unusual natural statistics. Mechanistically, the explanation for heterogeneity might lie in glomerulus-specific levels of GABA receptor expression (Root et al., 2008).

*Circuit Mechanisms: Connectivity between Glomeruli*

It is generally thought that specific connectivity between glomeruli is important for olfactory processing (Laurent, 2002; Lledo et al., 2005). Here we show that specific connectivity is not required to account for PN odor responses: good fits to data can be obtained by assuming all-too-all connectivity and uniform connectivity weights. We found that sparser connectivity can also generate good fits (Figure S8) because the responses of different ORN types are correlated with each other, and so pooling input from only a subset of glomeruli produces an effect similar to pooling total input. However, most individual *Drosophila* antennal lobe local neurons innervate the majority of glomeruli (Das et al., 2008; Lai et al., 2008), and this implies a comparatively dense pattern of interglomerular connections.

In the mammalian olfactory bulb, one local interneuron cannot connect all glomeruli. However, dense nonspecific connectivity could be implemented on a local scale. Nearby glomeruli in the bulb are almost as diverse in their odor selectivity as distant glomeruli (Soucy et al., 2009). Thus, the summed responses of local glomeruli might produce an inhibitory signal similar to the sum of all glomeruli. Alternatively, if mammalian ORN types are not as correlated in their odor selectivity as *Drosophila* ORN types are, then optimal connectivity might be sparse and specific (Fantana et al., 2008).

It should be noted that excitatory lateral connections coexist in this circuit with inhibitory ones (Olsen et al., 2007; Root et al., 2007; Shang et al., 2007). In this study, we found that the net effect of lateral input was always inhibitory. However, this does not imply that lateral excitatory connections make no contribution—only that they do not dominate.

*Cellular Mechanisms: Pre- versus Postsynaptic Inhibition*

Lateral inhibition in the adult *Drosophila* antennal lobe has a mainly presynaptic locus (Olsen and Wilson, 2008; Root et al., 2008). This raises the question of why it might be useful to implement inhibition pre- rather than postsynaptically. Our results suggest a novel answer. We show that lateral inhibition in this circuit produces input gain control rather than response gain control, and input gain control has some attractive properties. It is easy to see why presynaptic inhibition might produce input gain control: any inhibitory process that acts prior to the nonlinearity in the input-output function will tend to make it more difficult to reach saturation but will not change the level at which output saturates. The major nonlinearities in the intraglomerular transformation are short-term synaptic depression and the postsynaptic refractory period (Kazama and Wilson, 2008), whereas presynaptic inhibition is thought to modulate an earlier step, i.e., presynaptic calcium influx. In other circuits the mechanisms of normalization may be different, and may not involve GABAergic inhibition (Carandini et al., 2002; Freeman et al., 2002).

*Dynamics of Gain Control*

We found that increasing total ORN activity (by increasing the public odor concentration) made PN responses more transient. This result has parallels in other sensory modalities, where increasing stimulus intensity generally decreases neuronal integration times. For example, in the retina, increasing the luminance of a visual stimulus produces more transient responses in ganglion cells (Shapley et al., 1972; Enroth-Cugell and Shapley, 1973). In visual cortex, increasing the contrast of a periodic visual stimulus advances the phase of neural responses (Dean and Tolhurst, 1986). Similarly, increasing sound intensity narrows the integration time of auditory cortical neurons (Nagel and Doupe, 2006). These changes create an adaptive tradeoff that should maximize information transmission over a range of stimulus intensities (Atick, 1992). Long integration times should allow neurons to overcome the effects of noise when stimulus intensities are low, whereas short integration times should maximize temporal resolution of stimulus fluctuations when stimulus intensities are high. Our findings extend this principle to olfactory processing.
Adaptive changes in integration time have been recognized as a natural extension of normalization models. For example, if normalization is implemented by an increase in postsynaptic inhibitory conductances, then the resulting decrease in the postsynaptic membrane time constant would shorten the integration time (Carandini and Heeger, 1994; Carandini et al., 1997). However, in the Drosophila antenna lobe, lateral inhibition is largely presynaptic (Olsen and Wilson, 2008), so this mechanism is unlikely to apply. Instead, our results suggest an alternate mechanism: shorter integration times are likely due to increasingly rapid recruitment of lateral inhibition by increasingly intense affenter activity.

Limitations of the Model
First, our model is based on measurements from only a few glomeruli. In pilot experiments we explored other candidates, but we could not find private odors for these glomeruli. This reflects the constraint that the private odor must be selective even at concentrations high enough to approach $R_{\max}$.

Second, we have not modeled the dynamics of neural activity. Because the input data set for our model consists of ORN firing rates averaged over a 500 ms time period (Hallem and Carlson, 2006), our model is not able to consider finer timescales. ORN responses are themselves dynamical, and these dynamics depend on both the odor and the ORN (Hallem and Carlson, 2006). PN response dynamics are also characteristic of faster than ORN response dynamics (Bhandawat et al., 2007). Modeling these dynamics will require a more systematic understanding of these processes.

Finally, the usefulness of any transformation will depend on the decoder and the task. Our model decoders are inspired by the properties of real higher-order olfactory neurons. However, some aspects of our model decoders are unrealistic—for example, each pools input from all glomeruli. Unraveling the actual connectivity of the higher-order olfactory circuit should help us better constrain our models. Also, the tasks we set our decoders are probably easy compared to natural olfaction, which is complicated by turbulence and background odors. Understanding how these factors affect olfactory encoding should help us gain insight into the tasks this circuit has evolved to perform.

**EXPERIMENTAL PROCEDURES**

**Fly Stocks**
Fly stocks were kindly provided as follows: NPy221-Gal4, NP3062-Gal4, NP481-Gal4 (Kei Ito and Liqun Luo); Or92a-Gal4 (Leslie Vosshall); UAS-CD8GFP, UAS-CD8GFPp6 (Li), UAS-CD8GFPp6p6, Or22L+f74939 (see Figure S1), Or429+f74939 (see Figure S1).

**Electrophysiological Recordings**
The total number of observations in this study comprises 1299 ORN measurements, 225 LFP measurements, and 591 PN measurements (total n summed across all experiments). Each measurement represents the mean of four consecutive trials with the same stimulus. ORN spikes were recorded extracellularly from sensilla on the surface of the maxillary palp or antenna. The antennal LFP was recorded with an electrode in the body of the antennal funiculus. Whole-cell patch-clamp recordings were made from PN somata in current-clamp mode. Recordings were targeted to specific PNs by labeling them with GFP. See Supplemental Experimental Procedures for details.

**Ofactory Stimuli**
See Supplemental Experimental Procedures for details.

**Data Analysis**

**Quantifying Neural Responses**
Each cell was tested with multiple stimuli, typically with four trials per stimulus spaced 40-60 s apart. The response magnitude for each cell/stimulus combination was quantified as the trial-averaged spike rate during the 500 ms odor stimulus period, minus the trial-averaged baseline spike rate during the preceding 500 ms. To generate a peristimulus time histogram, we counted the number of spikes in 50 ms bins that overlapped by 25 ms. LFP recordings were quantified as the integral during the 500 ms odor stimulus period, minus the integral during the 500 ms preceding the stimulus. All these response measures were first averaged across trials within an experiment, and then reported as mean ± SEM across experiments.

**Fitting Input-Output Functions**
The input-output functions in Figure 1 were determined by fitting the private odor responses for each glomerulus to Equation 1. $R_{\max}$ and $s$ were free parameters. $R_{\max} = 170, 167, 163, and 144, and s = 16.3, 11.8, 12.4,$ and 44.8, for glomeruli DM4, DLS, VM7, and DM1, respectively. Equation 1 fits these data better than the logarithmic function used in previous studies (Bhandawat et al., 2007; Olsen and Wilson, 2008).

In Figure 3, each input-output function within a panel corresponds to a different concentration of pentyl acetate. Here we used Equation 2 for the input gain model and Equation 3 for the response gain model. The parameters $R_{\max}$ and $s$ were derived separately for VM7 and DLS from the fits in Figure 1 and were held constant across all concentrations of pentyl acetate. Thus, the only free variable in these fits was $s$.

In Equations 1–3, the input variables $ORN$, $PN$, and $s$ are raised to an exponent (1.5). We use this exponent because it provides the best fit to our data. We determined this by fitting the data in Figure 3 with different exponents between 1 and 2 in increments of 0.1. The mean squared error had a minimum for an exponent of 1.5 and 1.6 for glomeruli DLS and VM7, respectively. Choosing an exponent of 1.5 for VM7 produced only a slight decrease in fit quality and allowed a constant exponent to be used for all equations.

**Predicting PN Odor Responses Based on the LFP**
In Figure 4, PN responses to novel stimuli were predicted from Equation 2 on the basis of two variables: the presynaptic ORN response to that stimulus (ORN) and the value of $s$ corresponding to that stimulus. Values of $s$ were derived from the LFP response to each stimulus according to Equation 4. The relationship between $s$ and the LFP was obtained from the linear fit in Figure 3H ($m = 10.63$ for VM7 and 4.19 for DLS). Each LFP value in Figure 3H is the sum of the LFP response to one pentyl acetate concentration (Figure 2A, different for each curve) and the LFP response to the private stimulus alone (the same for each curve). Summation is reasonable because public and private odors do not activate the same ORNs, and because the LFP scales linearly with summed ORN firing rates (Figure S3). To fit a curve, we needed to represent the contribution of the private stimulus to the summed LFP with a single value, but in reality each curve was constructed with a range of the private odor concentrations, all of which elicit slightly different small LFP responses (Figure S1); for simplicity, we averaged the LFP measured for all these concentrations to estimate the contribution of private stimuli to the LFP.

**Modeling**

**Simulating PN Responses**
In Figures 5–7 we used the data from Hallem and Carlson (2006) to stimulate PN population codes. Because this data set includes only 24 of the 50 ORN types, we simulated only 24 glomeruli. Unless otherwise noted, we used the following parameters for all glomeruli: $R_{\max} = 165$ spikes/s and $s = 12$ spikes/s. To simulate the PN matrix without inhibition, we used Equations 2 and 6. The constant $m$ in Equation 6 was set to 10.63 for all glomeruli. The response gain PN response matrix was simulated using Equations 3 and 6 ($m = 0.164$). For all simulated PN responses, if the presynaptic ORN odor response was a negative value, then the response was set to zero. Population response magnitude (Figure 5C) was quantified for each stimulus as the norm of the response vector in 24-dimensional ORN or PN space.
where \( r_i \) is the firing rate of the \( i \)th ORN type or PN type.

**Decoding Simulated PN Responses with a Linear Classifier**

Each perceptron receives input from all 24 glomeruli. The perceptron classifies stimuli by computing a weighted sum on its inputs and responding if this sum crosses a threshold, \( c \). Its response is binary:

\[
\text{response} = \begin{cases} 
1 & \text{if } c \leq w_i r_f + w_{i+1} r_{f+1} + \cdots + w_{24} r_{f+24} \\
0 & \text{if } c > w_i r_f + w_{i+1} r_{f+1} + \cdots + w_{24} r_{f+24} 
\end{cases}
\]

where \( r_i \) is the response of the \( i \)th glomerulus and \( w_i \) is the weight of that glomerulus. The weights for each perceptron were derived using the normalized perceptron learning rule:

\[
w_{\text{new}} = w_{\text{old}} + \eta \frac{p - r_i}{||p||}
\]

where \( p \) is the norm of the training input vector, \( p \), is the input from \( i \)th glomerulus to the perceptron, and \( x \) is the difference between the perceptron’s output and target value. Additionally, we constrained the sign of the weights to be nonnegative. If the updated weight took a negative value this weight was reverted to its previous nonnegative value before presenting the next training input. The threshold \( c \) was constrained to be the same for all perceptrons within the same set and was adjusted during training so that the false hits rate was equal to the rate of false misses.

For each set of perceptrons, we generated 100 noisy training matrices by picking the appropriate matrix in Figure 5A and adding Gaussian noise to rate was equal to the rate of false misses.

within the same set and was adjusted during training so that the false hits
rate was equal to the rate of false misses.

For each set of perceptrons, we generated 100 noisy test matrices, generated in the same way as the high concentration.

concentrations of each odor represent 100-fold and 10,000-fold dilutions of this repeated 500 times).

500 independent networks (i.e., 100 training iterations, followed by 50 tests, See Supplemental Experimental Procedures.

Alternative Models of Gain Control and Odor Discrimination

See Supplemental Experimental Procedures.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes one table, eight figures, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.neuron.2010.04.009.

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**REFERENCES**


Divisive Normalization in Olfactory Population Codes
Shawn R. Olsen, Vikas Bhandawat, and Rachel I. Wilson
**ORN and LFP Recordings**

Flies were immobilized in the trimmed end of a plastic pipette tip. An antenna or maxillary palp was stabilized using glass capillaries or coverslips positioned using micromanipulators. The recording electrode was a sharp saline-filled glass capillary was inserted into a sensillum for ORN recordings, or inserted into the third antennal segment for LFP recordings. A sharp saline-filled glass capillary in the eye served as a reference electrode. Signals were recorded on an A-M Systems Model 2400 amplifier with a 10-MΩ headstage, low-pass filtered at 2 kHz and digitized at 10 kHz. Recorded ORNs were identified based on sensillum morphology and size, sensillum position on the antenna or palp, spike amplitude, spontaneous spike frequency, and odor tuning of all cells in a sensillum. These properties form an unambiguous signature of ORN identity. To sort spikes from the DM1 ORN, it was necessary to kill another ORN housed in the same sensillum using diphtheria toxin light chain (DTL), so these recordings were made in flies with the genotype *UAS-DTl/CyO;Or92a-Gal4*. Other ORN recordings were performed in the following genotypes: VM7, *NP3481,UASCD8GFP*; DL5, *NP3062,UASCD8GFP*; DM4, *NP3062,UASCD8GFP*. ORN recordings in Figure S1A were performed in *w* flies. For this figure, we recorded from a total of 223 ORNs, sampled randomly from all morphological classes (30 antennal large basoconic, 106 antennal small basoconic, 24 palp basoconic, 30 trichoid, 28 coeloconic). Of these 223 ORNs, 42 had spike waveforms which could not be sorted from the waveform of another ORN housed in the same sensillum; in these cases, the summed response was divided in half and each half assigned to one ORN. None of the unsortable sensilla showed a strong response to any private stimuli (range = -13.6 to 10.6 spikes/sec, interquartile range -3.8 to 0.2 spikes/sec).

For LFP measurements, we selected recording sites with the knowledge that the contribution of each ORN type to the LFP may depend on the recording site. We used an odor (ethyl acetate 10-8) that activates a single ORN type (DM1 ORNs) situated on the proximal end of the antenna to understand how the signal decays as a function of distance. We found that the signal decays to about 50% of the peak value as the recording site moved distally along the long axis of the third segment, with little decay of the signal along the short axis. Therefore, in order to weight proximal and distal ORN types roughly equally, we measured the LFP at one proximal site and one distal site, and then averaged these measurements together.

**PN Recordings**

The composition of the internal patch-pipette solution was (in mM): potassium aspartate 140, HEPES 10, MgATP 4, Na₂GTP 0.5, EGTA 1, KCl 1, biocytin hydrazide 13 (pH = 7.3, osmolarity adjusted to ~ 265 mOsm). The composition of the external saline was (in mM): NaCl 103, KCl 3, N-tris(hydroxymethyl) methyl-2-aminoethane-sulfonic acid 5, trehalose 8, glucose 10, NaHCO₃ 26, NaH₂PO₄ 1, CaCl₂ 1.5, and MgCl₂ 4. Osmolarity was adjusted to 270–275 mOsm. The saline was bubbled with 95% O₂/5% CO₂ and reached a final pH = 7.3. An Olympus BX51WI microscope with a 40x water-immersion objective and IR-DIC optics was used to obtain recordings under visual control. Recordings were targeted to specific PNs based on GFP visualization. We targeted 4 PN types in this study: DM1 in *NP5221-Gal4,UASCD8GFP*, DM4 and DL5 in *NP3062-Gal4,CD8GFP*, and VM7 in *NP3481-Gal4,UASCD8GFP*. Recordings were obtained with an A-M Systems Model 2400 amplifier in current-clamp mode (10-MΩ headstage), low-pass filtered at 5 kHz, and digitized at 10 kHz.

GABA receptor antagonists were prepared as concentrated stock solutions and a measured volume of stock was added to the saline perfusing the brain to achieve final concentrations of 5 µM (picrotoxin, Sigma) and 10 µM (CGP54626, Tocris). Occasionally these drugs caused the PN membrane potential to oscillate at ~2-4 Hz; when this occurred the recording was terminated.

**Olfactory Stimuli**

Test stimuli for VM7 PNs (Figure 4A,B) were the following monomolecular odors at 1:100 dilution: methyl salicylate, benzaldehyde, linalool, 1-octen-3-ol, 1-butanol, trans-2-hexenal, 2-heptanone, pentyl acetate, butyric acid, ethyl acetate, ethyl butyrate. We also tested 6 blend stimuli on VM7 PNs. Three blend stimuli were made by mixing methyl salicylate 10⁻², fenchone 10⁻¹, methyl acetate 10⁻⁸, trans-2-hexenal 10⁻⁸, propionic acid 10⁻⁵ with three concentrations of butanone (10⁻⁴ to 10⁻⁸). Three more blend stimuli were generated by adding
pentyl acetate $10^{-3}$ to each of the above blends. Test stimuli for DL5 PNs (Figure 4C,D) were the following monomolecular odors at 1:100 dilution: acetophenone, butanal, benzadehyde, butyric acid, 1-butanol, cadaverine, ethyl butyrate, ethanol, hexanal, linalool, methyl salicylate, 3-methylthio-1-propanol, 1-octen-3-ol, paraffin oil, valeric acid, pentyl acetate, 1-penten-3-ol.

Reported odor concentrations represent v/v dilutions in solvent and are 1:100 unless otherwise noted (always paraffin oil, J.T. Baker, VWR #JTS894, except for 3-methyl-thio-1-propanol, which was diluted 1:100 v/v in water, and ethanol, which was diluted 1:100 w/v in water). Odor stimulation was performed as described previously (Bhandawat et al., 2007) with modifications described here. As in previous studies, a constant stream of charcoal-filtered air (2.2 L/min) was directed at the fly throughout each experiment, and when triggered by a command pulse, a three-way solenoid valve redirected a portion (0.20 L/min) through the headspace of the odor vial for 500 msec. Thus all odors were diluted an additional 10-fold in air just before reaching the fly. In this study, in order to deliver odor blends, we added a second solenoid to direct a portion of the carrier stream (0.20 L/min) through a second odor vial. The two solenoids were triggered simultaneously, and they drew from the carrier stream at the same point. The two odorized streams both rejoined the carrier stream 15 cm from the end of the end of the delivery tube, which measured 3 mm in diameter and was positioned 8 mm from the fly. We made fresh dilutions in solvent every five days, except odors with high vapor pressures (methyl acetate and ethyl acetate) which were replaced every three days. These two odors were also made up in a larger volume (5 mL vs 2 mL), so as to deplete a smaller fraction of the odor molecules in the headspace on each trial.

**Data Analysis and Modeling: alternative models of gain control**

In addition to evaluating the input gain control and response gain control models (Figure 3), we also evaluated three other models of gain control. First, we evaluated two subtractive models of inhibition. In one,

$$P N = R_{\text{max}} \left( \frac{(O R N - s)^{1.5}}{(O R N - s)^{1.5} + \sigma^{1.5}} \right)$$

This produced a poor fit (mean squared error = 846 and 149 spikes$^2$/sec$^2$ for VM7 and DL5, compare to Figure 3G). The second subtractive model was a downward shift in the input-output function:

$$P N = R_{\text{max}} \left( \frac{O R N^{1.5}}{O R N^{1.5} + \sigma^{1.5}} \right) - s$$

This also produced a poor fit (mean squared error = 245 and 80 spikes$^2$/sec$^2$ for VM7 and DL5).

Finally, we evaluated a model where we held $R_{\text{max}}$ and $\sigma$ constant across all concentrations of pentyl acetate and instead we allowed the exponent in Equation (1) to vary across pentyl acetate concentrations. This produced the poorest fit to the data.

**Data Analysis and Modeling: an alternative model of odor discrimination**

In an additional analysis, we allowed perceptrons to have both positive and negative weights, and we allowed each perceptron to have a different threshold. To determine the weights and the thresholds, we used either a support vector machine with a linear kernel or else, in a separate analysis, Fisher’s linear discriminant analysis. Performance rates were always higher for these perceptrons than for sign-constrained perceptrons. We do not describe these results in detail because (1) these perceptrons are less biologically realistic than sign-constrained perceptrons, and because (2) their higher performance rates leave less dynamic range for seeing the effects of the transformations we describe. However, we confirmed that our main conclusions are still true for these networks: the input gain model always performs better than the model without lateral inhibition, and it also outperforms the response gain model. The only difference was that the effects of these transformations were smaller, and the distributions of errors in the confusion matrices were different.

It should be noted that the performance of perceptrons trained and tested with the response gain model can be improved by changing the overall level of inhibition or changing the form of the relationship between $s$ and total ORN activity. We did not explore these alternatives systematically because our goal was simply to compare the versions of these two gain models that were the best fits to our data.
Table S1: Candidate “private” odor stimuli.

| Odorant Receptor | 2a | 7a | 9a | 10a | 19a | 22a | 23a | 33b | 35a | 42a | 42b | 43a | 43b | 47a | 47b | 49b | 59b | 65a | 67a | 67c | 82a | 85a | 85b | 85f | 88a | 98a |
|------------------|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| trans-2-hexenal  | 10^-6 | 6   | 23  | -3  | -1  | 0   | 2   | 4   | 1   | 2   | 0   | 2   | -1  | -5  | 3   | 1   | 4   | -8  | 1   | -8  | -2  | 8   | -10 |
| ethyl acetate    | 10^-8 | 4   | 0   | 3   | -5  | 10  | -4  | 6   | -7  | 6   | 3   | -3  | 5   | -2  | 1   | 1   | 0   | -6  | -5  | 2   | -5  | -1  | 5   | -2  |
| ethyl acetate    | 10^-6 | -1  | 14  | 1   | -2  | 4   | 2   | 0   | 0   | -3  | 1   | -1  | -1  | 1   | -1  | 1   | -1  | 0   | 2   | 0   | 0   | 0   | -4  | -5  | -1  | -4  | -3  | -2  | -5  |
| methyl acetate   | 10^-2 | -1  | 4   | 6   | 14  | -3  | -4  | 1   | -1  | 56  | 7   | 3   | 10  | 1   | 0   | 2   | 0   | 0   | -4  | -5  | -1  | -4  | -3  | -2  | -5  |
| 2-butanone       | 10^-2 | -5  | 14  | 24  | 9   | -13 | 54  | 2   | 15  | -6  | 0   | 0   | 12  | 22  | 25  | -21 | -5  | 269 | -3  | 7   | 7   | 15  | -10 | 29  | 12  | 14  | 4   |

To identify “private” stimuli which activate just one ORN type, we first examined published data describing the spiking responses of many ORNs to a large panel of odor stimuli (Hallem & Carlson, 2006). Based on this data (reproduced in this table), it appeared that trans-2-hexenal, ethyl acetate, and methyl acetate were good candidates for “private” odors. In addition, our pilot experiments showed that 2-butanone strongly activated Or42a-expressing ORNs (VM7 ORNs) at low concentrations, suggesting this odor might also be a good candidate (see table).

Odorant receptors are listed in the top row of the table. Entries in the table represent mean firing rates, measured in units of spikes/sec over the 500-msec odor stimulus period, and averaged over several measurements in different flies. All entries in the table are from Hallem & Carlson (2006), except the three values in bold italics, which were measured by us. An open symbol (◦) indicates that no data is available. Strong responses indicating candidate receptor-odor pairs are highlighted in color.
Figure S1: Evidence that “private” odor stimuli activate mainly a single ORN type

A. Histograms show the distribution of responses in non-cognate ORNs to the four private odors at the highest concentrations used in this study. Note that these stimuli evoke almost no response from non-cognate ORNs. The vast majority of responses were essentially zero (median = -0.25 spikes/sec, 10th percentile = -3.1 spikes/sec, 90th percentile = 5.2 spikes/sec). Here we recorded randomly from 223 ORNs, targeting all morphological types to ensure good sampling. The four cognate ORN types corresponding to these private odors were identified by physiological and morphological criteria, and their responses to their private odors were excluded from this sample. See Supplemental Experimental Procedures for details.

B. By contrast, the “public” odor (pentyl acetate) evokes widespread activity at the highest concentration used. C. LFP responses in the antenna and the palp to the four private odors. In two cases, a mutation was available in the odorant receptor expressed by a cognate ORN (Or42b and Or42a), and here we verified that the LFP response to the corresponding private odor is virtually abolished by the mutation (see Experimental Procedures). Note that the cognate ORNs for the first three of these odors are located in the antenna (Or7a/DL5, Or42b/DM1, Or59b/DM4). For these odors, antennal LFP responses are small and palp responses are zero, implying that mainly a single antennal ORN type is active. Cognate ORNs for the last odor (2-butanone) are located in the palp (Or42a/VM7), where LFP responses to the private odor are larger, presumably because VM7 ORNs are a large fraction of all palp ORNs.

Note that the four “private” stimuli are not strictly private at the highest concentration we used. For example, it is clear the 2-butanone (10^{-4}) evokes a small amount of activity in antennal ORNs. In choosing the concentrations of “private” odors used in this study, we were constrained by the need to drive the cognate PNs for these odors to a level approaching saturation, and this necessitated some compromises for the highest of these concentrations. Nevertheless, the fact that a few non-cognate ORNs respond weakly to these stimuli does not affect our major conclusions.
Figure S2: Pentyl acetate does not affect VM7 or DL5 ORNs.
A. The “public” odor pentyl acetate does not affect the response of VM7 ORNs to their private odor, 2-butanone. Recordings from VM7 ORNs show the response to either 2-butanone alone or 2-butanone blended with pentyl acetate ($10^{-3}$, the highest concentration used in this study). Bar indicates odor delivery period. Each peristimulus-time histogram is a mean of 4-8 recordings, ± SEM.
B. Average firing rate over 500-msec odor stimulus window.
C-D. Analogous to (A-B) for DL5 ORNs. Private odor is trans-2-hexenal. Each PSTH is a mean of 5-6 recordings.
Figure S3: Antennal LFP is linearly related to total ORN spike rate.

A. LFP recordings from the antenna are correlated with the summed response of all 24 ORN types. ORN data is from Hallem and Carlson (2006). Each point is a different odor. The slope of the fitted line is (1/190) mV·sec²/spikes.

B. The antennal LFP summates linearly, at least within the response regime we are investigating in this study. We show this by measuring LFP responses to 6 stimuli that activate largely non-overlapping sets of ORNs, and also responses to three blends of these stimuli. LFP responses to the blends were predicted by summing the LFP responses to the individual components. Odors used were ethyl acetate (10⁻⁶), methyl acetate (10⁻⁶), trans-2-hexenal (10⁻⁶), 2-heptanone (10⁻⁶), 1-hexanol (10⁻⁶), and methyl salicylate (10⁻⁴). Each point is a separate experiment. Red points are responses to a blend of all 6 odors; green points are responses a blend of 5 odors (methyl acetate was held out); gray points are responses a blend of ethyl acetate (10⁻⁶) and trans-2-hexenal (10⁻⁶).
Figure S4: The suppression factor $s$ and the LFP are linearly related.

Here, the solid circles and fitted line are reproduced from Fig. 3h. The open symbols represent values computed from the data in Fig. 4, as follows. For each of the test stimuli in Fig. 4, we obtained the value of the suppression factor $s$ by taking the measured PN firing rate and the measured ORN firing rate, and plugging these into Equation (2). For each test stimulus, we have also measured the antennal LFP. Plotting $s$ versus the LFP response reveals a significant linear correlation between $s$ and LFP ($p<0.01$ for each glomerulus, Pearson’s correlation, all data from Figs. 3 and 4 pooled together for each glomerulus). Moreover, this figure shows that the slope of this relation for the open symbols is well-described by the line fitted to the solid symbols. A sublinear function (exponential or hyperbolic) would not be a good description of this data.

In essence, this analysis explains why Equation (2) and Equation (4) together produce such good predictions of PN responses (Fig. 4): $s$ is indeed a linear function of the LFP, and this can be used to predict how much lateral inhibition suppresses the PN’s response to its direct ORN inputs.
Figure S5: Odor intensity is the stimulus feature that accounts for the largest share of the variance in ORN responses.
We performed PCA on the 24-dimensional ORN response vectors obtained from Hallem & Carlson (2006). Here we plot scores on the first PC versus the total ORN spike rate evoked by that stimulus (each point is a different stimulus). This plot shows that scores on the first PC are highly correlated with the total number of ORN spikes evoked by that stimulus. This indicates that PC1 is essentially a proxy for the intensity of the stimulus from the perspective of the ORN population. This is why normalizing for intensity reduces correlations among PNs: stimulus intensity is a large source of covariance in this population.
Figure S6: Modeling noise in PN responses.

We used data collected for a previous study (Bhandawat et al., 2007) to derive a model of how trial-to-trial variability depends on mean firing rate. In collecting this data set, each odor was presented for a block of 5 trials per cell. We measured the firing rate of individual recorded PNs over the entire 500 msec odor presentation window for each trial. For each block of trials, we computed both the mean firing rate and the standard deviation (SD) of the firing rate. Each point corresponds to a different block of 5 trials and a different cell/odor combination (n = 787 blocks of trials). The blue line shows SD values binned by mean (bin width = 20 spikes/sec). We fit this data with an exponential function (red line):

$$\text{SD} = (9.5 \text{ spikes/sec}) - (7.2 \text{ spikes/sec}) \cdot e^{-\text{mean}/(76 \text{ spikes/sec})}$$

This function was used to simulate noise in PN responses (Figs. 6-7). Each entry in the matrix was treated as the trial-averaged mean firing rate for that stimulus-PN combination. For each mean firing rate, we used this equation to define a SD. We drew noise randomly from a Gaussian distribution with having this SD and a mean of zero.
Figure S7: Correlations between stimuli are relatively unaltered in our model of antennal lobe transformations.

Each 176x176 matrix shows all pairwise correlation coefficients between stimuli. The first matrix shows correlations between stimuli in the ORN data (i.e., no transformation). Note that none of our simulated antennal lobe transformations has much effect on these correlations. The intra-glomerular transformation slightly increases correlations because it tends to push PNs toward saturation, but this effect is small. The two gain control models have little effect on correlations between odors because they affect all glomeruli uniformly in a manner that does not depend on the odor tuning of each glomerulus. Thus, each population odor response tends to be scaled down by some amount that depends on the odor, but the correlations between different odor-evoked glomerular patterns are not changed substantially. In geometric terms, the angles between the 176 (24-dimensional) odor response vectors are relatively unchanged. What does change is the norm of these vectors, such that all vectors now have more equal norms (Figure 5). This reduces correlations between glomeruli – but not correlations between stimuli.
Throughout this study, we have assumed that all ORNs contribute equally to the normalization pool. Here we examine how prediction quality changes if we instead assume sparser connectivity. To investigate this, we allowed glomeruli to make differing contributions to the normalization pool by replacing the sum in Equation (6) with a weighted sum:

$$s = R \sum w_i r_i / 190 \text{mV} \cdot \text{sec}^2/\text{spikes}$$

We then manipulated the vector $w$ of all the glomerular weights ($w_1, w_2, \ldots , w_{24}$) and examined how this affected the predictions of the model.

A. Responses to 16 odors in 24 ORN types (data from Hallem & Carlson, 2006). These 16 odors are the same as the set used to test predictions of the DL5 model (Fig. 4).

B. Inhibitory input to DL5 evoked by 16 odors is shown as a vector $s$ with 16 elements. This vector represents the value of the suppression factor $s$ for each odor (maximum inhibition in red, and minimum in blue). This vector is obtained by multiplying the ORN response matrix $R$ by the 24-dimensional weight vector $w$ and scaling by a constant. Two cases are shown: all-to-all connectivity (all weights =1) and sparser connectivity (50% of weights randomly zeroed). (In order to keep to the total level of inhibition constant when zeroing some weights, we scaled the non-zero weights by a factor of $24/N$, where $N$ is the number of non-zero weights.) Because ORN responses are correlated with each other, the inhibition vector is similar for these two cases: weak stimuli (top) typically produce weak inhibition while intense stimuli (bottom) produce strong inhibition.

C. We used the equation above to determine the value of the suppression factor $s$ for each odor. We then used Equation (2) to predict PN responses and computed the mean squared error of the prediction. For each connection number we ran 100 simulations. Each symbol represents the mean squared error for one iteration of the simulation.

Note that, on average, predictions degrade as more weights are randomly zeroed. Nevertheless, it is possible to find sets of weights that generate good predictions. This is not surprising because there are many degrees of freedom in this fit. Indeed, these sparse models overfit the data, because we can also obtain good predictions when we shuffle the odor labels on the ORN response vectors before fitting. In sum, the predictive power of our model does not place strong constraints on connectivity between glomeruli. What is notable is that good fits are obtained with the simplest model, where all glomeruli make an equal contribution.