CHAPTER 8

Gain control in early olfactory circuits

Abstract

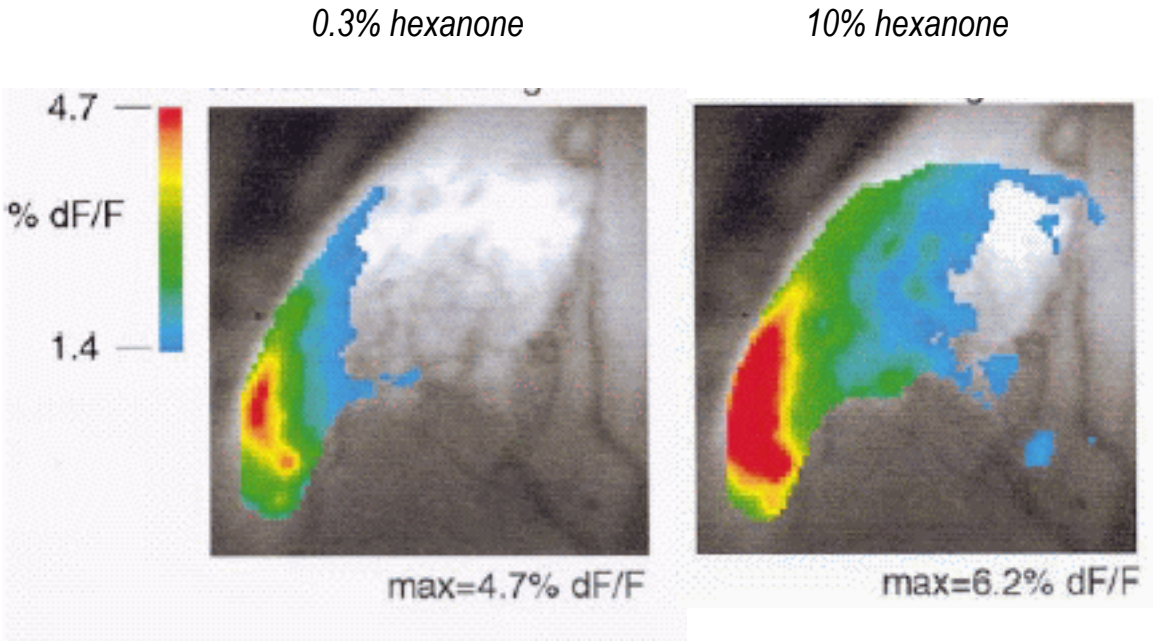
It has been known for some time that an increase in odor concentration causes a monotonic increase in the active area of the vertebrate olfactory bulb and insect antennal lobe as measured with calcium imaging, suggesting that downstream targets may have to deal with a large dynamic range of activity levels. On the other hand, response of mitral cells to increasing odor concentration has traditionally presented a less clear picture, with non-monotonic changes in firing rate. How are these pictures of input and output reconciled? Here, we record intracellularly from locust PNs and show that the mean firing rate across PNs is relatively invariant to odor concentration, due to a dynamic balance of excitation and inhibition acting as a gain control: as odor concentration increases, inhibition is strengthened in parallel to excitation. This effect, which is missed by calcium imaging techniques, may provide a solution to the conundrum of how complex odor blends do not evoke the percepts corresponding to all of their components, but rather a different percept altogether (Laing and Francis, 1989) despite the fact that receptors' responses are monotonic in concentration: the increase of inhibition with increasing concentration may allow detectors to be inhibited with the addition of additional blend components, allowing for cells to be tuned to particular odors. These may constitute dual roles of slow inhibition, whose role is controversial (Laurent, 2000), in the antennal lobe: tuning PN responses to respond specifically to particular odors, exhibiting lower responses to supersets of their preferred stimuli, and gain modulation, to keep PN firing rates within PNs' dynamic range and that of the decoders.

Introduction

The fundamental role of the olfactory system is, of course, to detect and recognize odors. Because information for rapid action is ultimately encoded in the nervous system by action potentials, there must be sets of neurons in the olfactory system which fire selectively in response to particular odors. Responding selectively to an odor, say apple, requires, in turn, responding to the presentation of apple while, at the same time, *not* responding to blends of apple with something else. There is behavioral evidence that animals indeed fail to recognize the components of complex odor mixtures (Laing and Francis, 1989).

Yet odorant receptor proteins have monotonic response functions of concentration: the higher the odorant concentration, the higher the proportion of receptors bound, and the larger the signal (Malnic et al., 1999). The number of olfactory receptor neurons (ORNs) responding to a given odorant increases with increasing concentrations of ligand (see Fig. 8.1, and Cinelli et al., 1995; Friedrich and Korsching, 1997; Joerges et al., 1997; Rubin and Katz, 1999; Wachowiak et al., 2000; Wachowiak and Cohen, 2001; Ziesmann et al., 2001). Some ORNs display a steep dependence of the mean instantaneous spike frequency (MISF) on stimulus strength saturating within a 10-fold increase in odor concentration (Ziesmann et al., 2001). Other ORNs display dose-response curves with a dynamic range covering concentrations of several orders of magnitude (Ziesmann et al., 2001).

Furthermore, optical imaging studies have shown that activity in the vertebrate olfactory bulb and its homologue in insects, the antennal lobe, increases monotonically with increasing concentration (Galizia and Menzel, 2001; Meister and Bonhoeffer, 2001). The dependency of the input to the olfactory bulb on odor concentration has been characterized with a simple formalism for ligand bind-



The response of olfactory receptor neurons in the three-toed box turtle, measured with Calcium Green-1 dextran 10 kD, is not concentration invariant, but rather increases with increasing concentrations (from Wachowiak et al., 2000).

ing (Holy et al., 2000; Meister and Bonhoeffer, 2001). If the olfactory system responds with increasing activity every time an odor is added, however, how does the system achieve odor-selectivity, which requires *not* responding to blends that include a response-eliciting component?

The antennal lobe, the insect analog of the vertebrate olfactory bulb, is the site of projection of olfactory receptor neurons. In locusts, it forms a compact (830 output neurons, or PNs, the insect analog of mitral cells), complete and dynamic representation of odors (Laurent et al., 2001). In order to characterize both excitatory and inhibitory inputs to PNs as well as PN output, I carried out intracellular recordings from PNs in the antennal lobe of awake locusts, while presenting series of 1-second-long odor puffs of varying concentrations using a computerized delivery system (see Chapter 6). Simultaneous recordings were carried out of the local field potential (LFP) in the mushroom body, the target of PNs.

Results

The field potential represents a measure of the population activity of synchronized PN assemblies (refs Laurent 94). The LFP showed increased power in the 13-30 Hz band during odor responses for all concentrations tested (Fig. 8.2a-b). The LFP's peak frequency remained constant across concentrations at 16-18 Hz (median peak frequency across trials for t=0-1 s was not significantly different for 2% concentration than for 100% concentration, p>0.4, Wilcoxon ranksum test, Fig. 8.2e).

Increasing odor concentration caused the peak-to-peak amplitude of the field potential during odor responses to increase, except for the highest concentration step, at which saturation, or even a slight decrease in LFP amplitude, occurred ($p<<10^{-6}$, n=133 PN-odor pairs, see Methods, Fig. 8.2a,c,d). This dependency was present both for naive animals (coefficient of correlation = 0.52, $p<<10^{-6}$, Pearson test and univariate Anova, n=133 PN-odor pairs) and for those that had been previously exposed to higher concentrations of the odor tested (coefficient of correlation = 0.61, $p<<10^{-6}$, Pearson test and univariate Anova, n=104 PN-odor pairs) (see Chapter 9). This was also reflected in an increase in the LFP's power in the 13-30 Hz window (Fig. 8.2a). This prompted the question of whether the increase in the LFP power was caused by an increase in PN firing rates, by a tightening in PN synchronization, or both.

Surprisingly, although mean PN firing rates increased in naive animals as a function of concentration (rate of minimum concentration tested, 2% of saturation, was not significantly different than that for maximum concentration, 100% of saturation; p<0.008, Wilcoxon ranksum test, n=152 PN-odor pairs), PN firing rates were not significantly affected by odor concentration after exposure to a higher concentration (rate of minimum concentration tested, 2% of saturation, was not significantly different than that for maximum concentration, 100% of saturation; p>0.8, Wilcoxon ranksum test, n=154 PN-odor pairs, Fig. 8.3) (see Chapter 9).

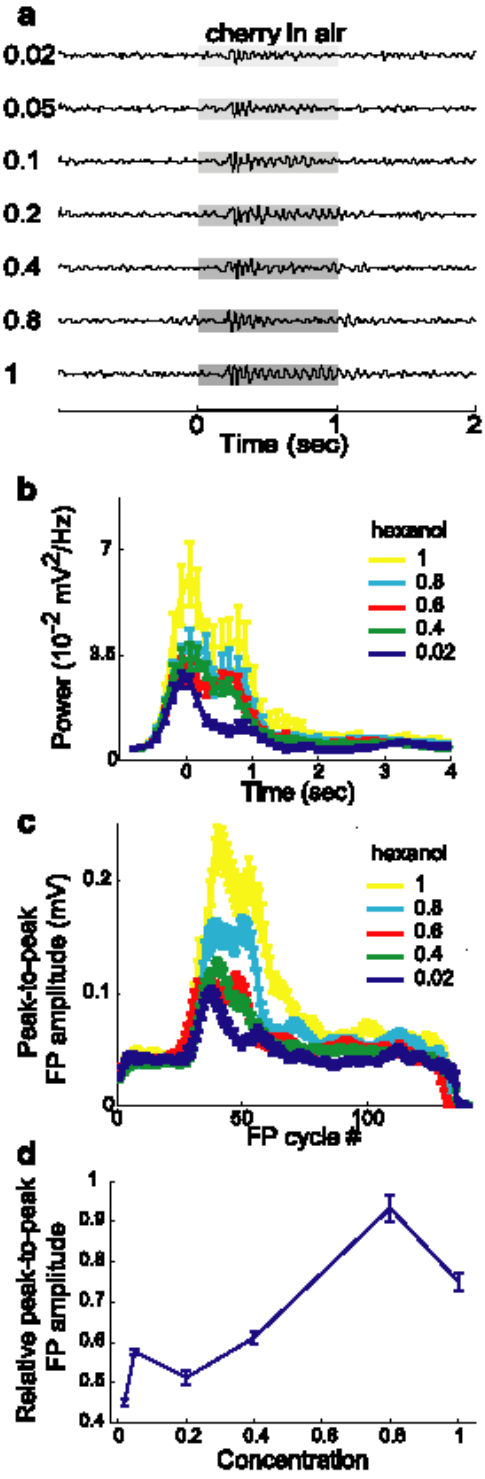
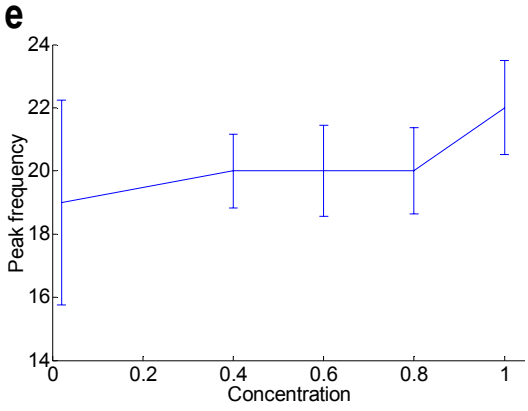


Figure 8.2 The local field potential's (LFP) peak-to-peak amplitude and power in the 13-30 Hz band increase as a function of concentration. a, LFP traces during exposure to a 1 sec pulse of varying concentrations, presented at t=0-1 s. **b,** Median power and s.e.m. in the 13-30 Hz band as a function of time and concentration for a typical PN-odor pair. Power was evaluated for 500-ms sliding windows. odor was presented at t=0-1 s. **c,** Median peak-to-peak amplitude (and s.e.m.) for every LFP cycle of the PN-odor pair in (b). **d,** Median peak-to-peak amplitude and s.e.m. averaged over cycles #30-80 and 133 PN-odor pairs.Cycle #1 was 1s before odor onset. e, Median peak frequency in the 0-100 Hz band for t=0-1 s for the PN-odor pair in (b) and (c).



A more detailed analysis of the intracellular recordings revealed that the lack of an effect of concentration on mean firing rate hid conflicting effects of concentration on excitation and inhibition. odor responses increased in contrast relative to baseline with increasing concentration. Increasing concentrations strengthened excitatory responses ($p<10^{-4}$, n=62 PN-odor pairs, see Methods), lengthened inhibitory responses (14 of 19 inhibitory responses were longer for higher concentrations, and mean duration was higher for higher concentrations, $p<0.003$, Wilcoxon ranksum test) and deepened inhibitory responses (18 of 19 inhibitory responses were more hyperpolarizing for higher concentrations, and mean hyperpolarized potential was more negative for higher concentrations, $p<<10^{-6}$, Wilcoxon ranksum test) of subthreshold inhibitory periods in naive animals (see Methods) (Fig. 8.4). After priming, the length of inhibitory responses was not significantly different for different concentrations ($p>0.05$), but inhibition remained significantly more hyperpolarizing for higher concentrations ($p<10^{-3}$). After priming with high concentrations, then, the strengthening of excitatory and inhibitory responses balanced each other out, leaving no net change in mean firing rates across PN assemblies as a function of concentration (Fig. 8.3).

The effect of concentration on the temporal response patterns of PNs was complex and varied for different PNs. All changes observed with increases in concentration could be explained by a strengthening of excitation, a strengthening of inhibition, or both. In no case did I observe a response present at any given concentration disappear at higher concentrations with no concomitant increase in excitation or inhibition. Some of the most typical responses are shown below. In some PNs, excitation and inhibition were strengthened approximately equally, and thus showed the same approximate temporal response patterns across the range of concentrations to which they responded (Fig. 8.5). In others, the duration of inhibitory responses increased with increasing concentration (Fig. 8.6). This systematically increased the latency of excitatory responses following the inhibition period. In PNs which exhibited early excitatory responses and no inhibition, the excitation

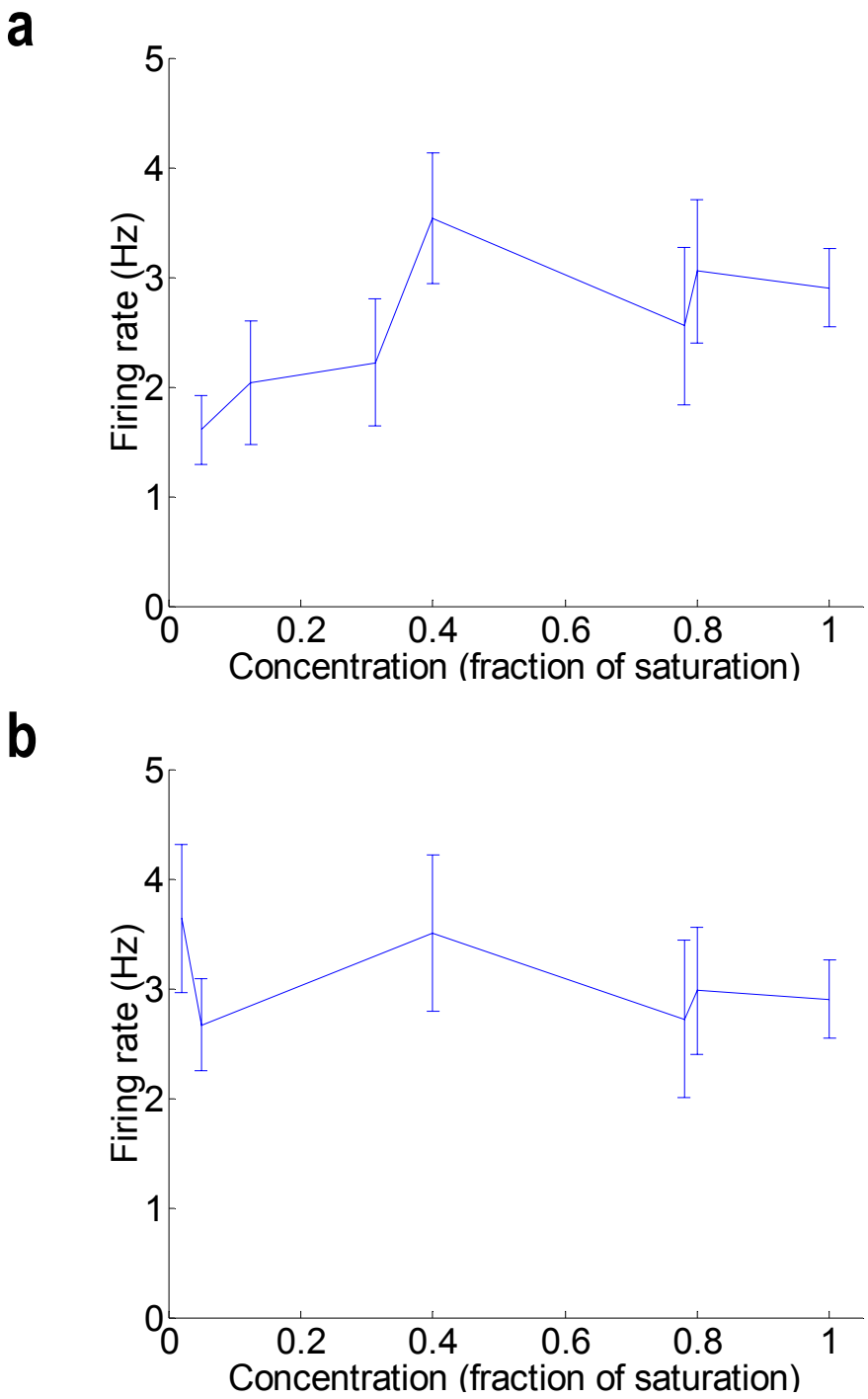


Figure 8.3 PN mean firing rates in naive animals increase slightly with concentration (a) but become independent of concentration after exposure to higher concentrations (b). Mean firing rates and s.e.m. for t=0-3 sec after odor presentation, averaged over 152 (a) and 154 (b) PN-odor pairs. S.E.M.s are large due to the heterogeneity of PN response patterns; the plot illustrates the overall average tendency of PNs to keep firing rates relatively constant over 2 orders of magnitude of concentration.

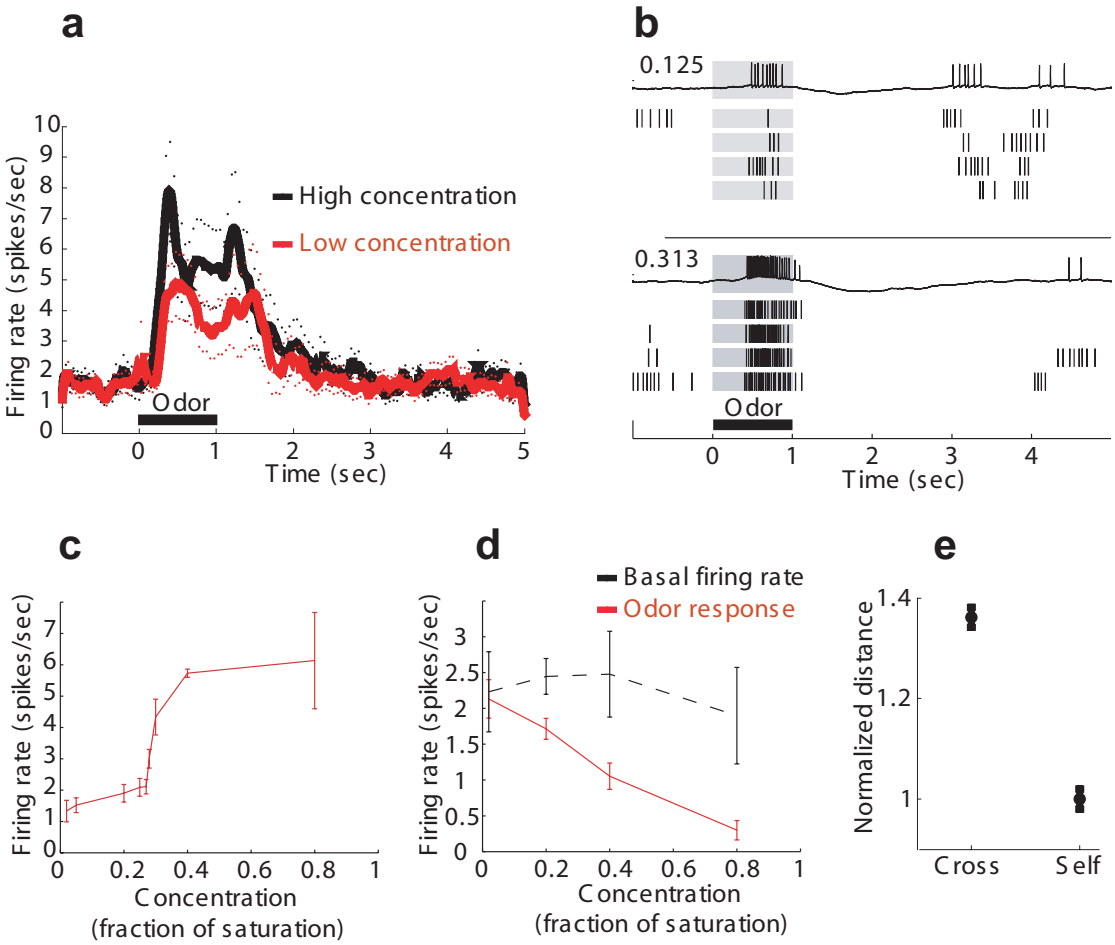


Figure 8.4 Response strength increases with concentration. a) The firing rate of PNs' excitatory responses increases with increasing odor concentration (maximum rates for higher concentration significantly higher than maximum rates for lower concentration, $p<10^{-4}$, n=62). Peri-stimulus time histogram (PSTH) of the mean and s.e.m. over all trials for 62 cell-odor pairs containing only excitatory responses, smoothed with a Gaussian (25 ms SD), for the highest and lowest nonzero concentration tested for each cell/odor pair. S.E.M.s are large due to the heterogeneity of PN response patterns. **b)** PN excitatory and inhibitory responses become more intense with increasing odor concentration. One PN's response to cherry over multiple trials at concentrations of 12.5% (top) and 31.3% (bottom). **c)** Another PN's response to citral as a function of concentration (mean firing rate and standard error of the mean, calculated from t=0.1-3.1 sec). **d)** Mean firing rate (and s.e.m.) of the PN in C as a function of concentration during the inhibitory response to apple (t=0.1-3.1 sec) and background activity during the 1-second period preceding odor presentation. **e)** Responses to different concentrations of the same odor are significantly different from each other ($p<<10^{-6}$, Distance test –see Methods—, n=62 cell-odor pairs). Cross: mean *difference between* low and high concentration series (and s.e.m.). Self: *variability within* series.

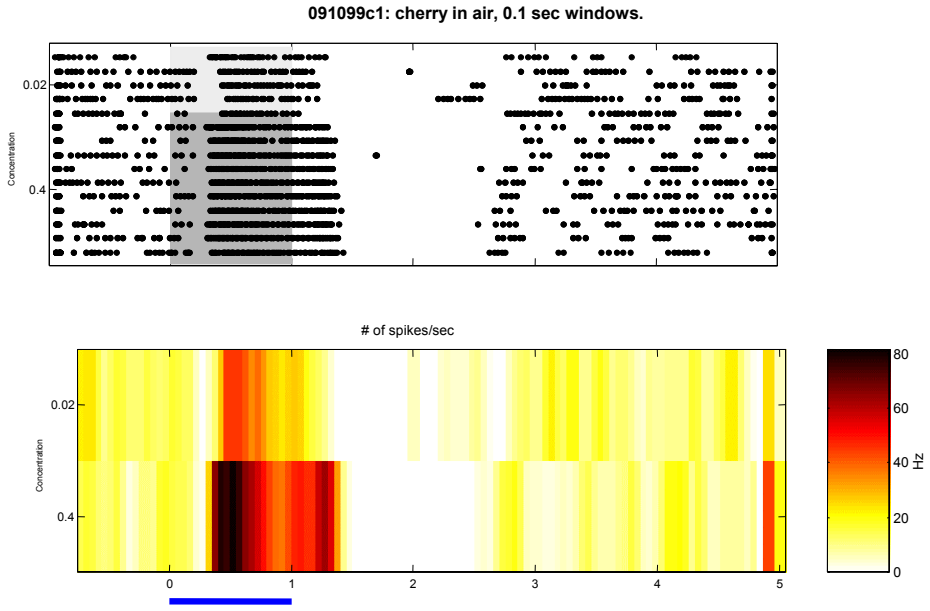


Figure 8.5 A PN's response to 2% and 40% of saturated cherry vapor. a) Spike rasters. **b)** PSTHs. Both excitatory and inhibitory responses are more pronounced at the higher concentration.

was usually strengthened, decreasing its latency and increasing firing rates (Fig. 8.7). Other PNs were recruited to an odor's representation for higher concentrations only, exhibiting excitatory and/or inhibitory responses absent at lower concentrations (Fig. 8.8). Concentration tuning (Kauer, 1974) was not found. It is conceivable that over larger concentration ranges, the strengthening of excitation could predominate at lower concentrations, followed by a predominant strengthening of inhibition at higher concentrations; this combination would produce an excitatory response only at intermediate concentrations, as described by Kauer for concentration tuned neurons (Kauer, 1974).

In contrast to the complex effects of concentration on PN firing rates, PN synchronization to the LFP, measured as the fraction of spikes during the 3-second period following presentation of odor whose phase with respect to the LFP fell within 3/8ths of a cycle of an LFP peak (from pi/2

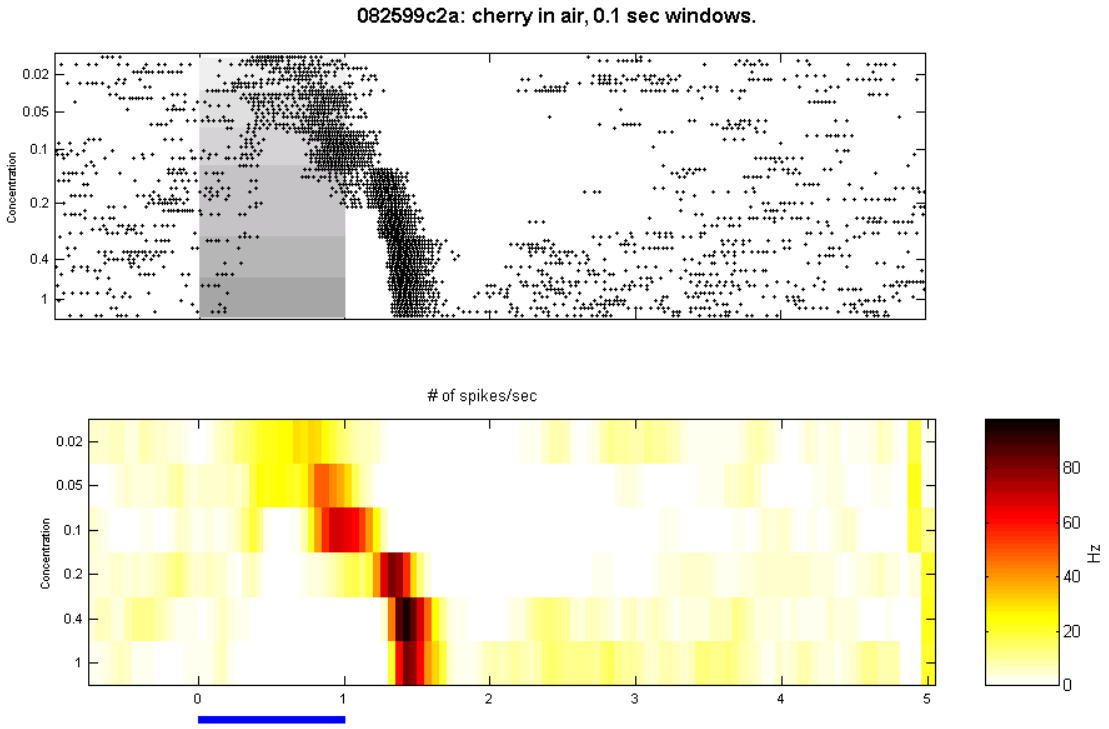


Figure 8.6 Increasing concentration often lengthens periods of hyperpolarization, increasing the latency of post-inhibition excitatory responses. a) Spike rasters**. b)** PSTHs.

before a peak to pi/4 after), increased significantly with increasing odor concentration (synchronization was significantly higher for maximum concentration (100% saturation) than for minimum concentration used (2% of saturation), $p<3x10^{-4}$, Wilcoxon ranksum test, n=144 PN-odor pairs, see Figs. 7.3 and 7.5). This effect was present in naive animals and was robust to exposure to higher concentrations (compare Fig. 7.5a to 7.5b).

In summary, we have demonstrated that odor-induced synchronization of PNs, measured as an odor-induced increase in the 20 Hz band of the field potential (Laurent and Naraghi, 1994; MacLeod and Laurent, 1996; Stopfer et al., 1997), is robust across a range of concentrations spanning two orders of magnitude. Second, we showed that increased odor concentrations strengthen the response of synchronized PN assemblies, as seen in an increase in the power and amplitude of

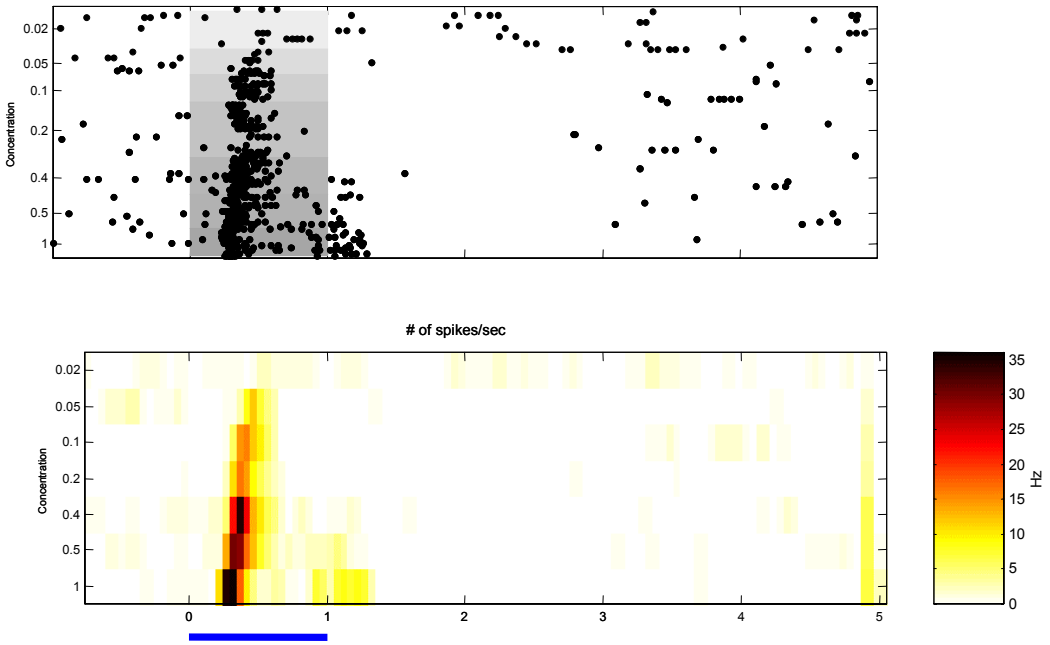


Figure 8.7 Increasing concentration often reduces the latency and increases the firing rate of early excitatory responses. a) Spike rasters. **b)** PSTHs.

the LFP. Third, we showed that these changes are not underlain by corresponding changes in mean firing rates, for both excitatory and inhibitory responses are strengthened and these effects balance each other out on average after exposure to high concentrations. Instead, the changes in the LFP as a function of concentration were primarily caused by a change in the degree of synchronization between PNs and the LFP. Finally, we explained the seemingly complex changes seen in PN temporal response patterns as combinations of two underlying tendencies, that of the strengthening of excitatory and inhibitory responses alike with increasing concentrations. This simple trend also provides an explanation for the concentration tuning observed by other workers in the olfactory bulb.

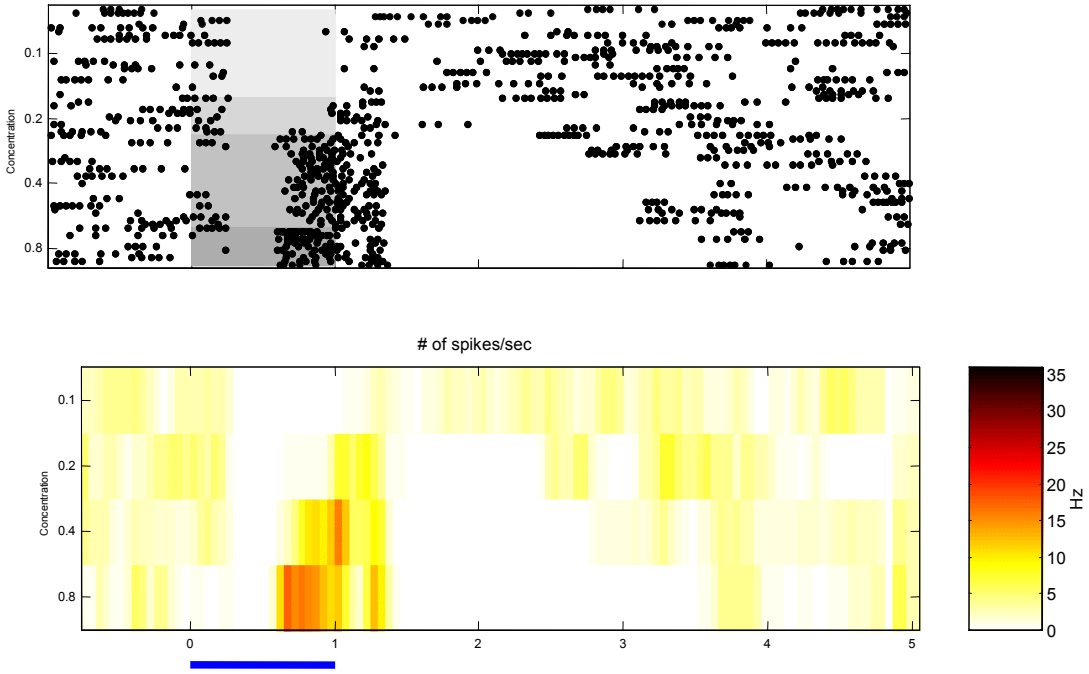


Figure 8.8 Some PNs get recruited to the odor representation at higher concentrations only. Note that both excitatory and inhibitory responses appear as concentration is increased. a) Spike rasters. **b)** PSTHs.

Discussion

Our results have several functional implications. First, they suggest that the odor-induced synchronization of assemblies of projection neurons in the antennal lobe of the locust (Laurent and Davidowitz, 1994; Laurent and Naraghi, 1994) is not restricted to high concentrations but rather extend over at least two orders of magnitude of concentration.

Second, the demonstration that the LFP was reliably strengthened with increasing odor concentrations suggests that odor concentration is encoded in the degree of synchronization of PN assemblies (see Chapter 7).

Third, the demonstration that both excitation and inhibition are strengthened by increasing

concentrations suggests that odor concentration is likely not encoded by a rate code, but rather by one involving identity and timing of spikes across neuronal assemblies. The finding that synchronization between PNs and the LFP increases with increasing odor concentration is consistent with this hypothesis. Of particular interest is the fact that this synchornization code for concentration endures after priming (see Chapter 9). This suggests that although priming enhances concentration invariance, this does not come at the expense of concentration information, which is maintained in the degree of synchronization across PN assemblies.

Fourth, the stark contrast between our results using intracellular recordings and those of optical imaging of the insect antennal lobe and vertebrate olfactory bulb brings into question the validity of optical imaging as a tool to study the output of neuronal assemblies. Recently, the source of optical imaging signals has been brought into question (Meister and Bonhoeffer, 2001; Galizia and Menzel, 2001). It has recently been suggested that calcium imaging signals may represent an overestimation of the output of the olfactory bulb (Galizia and Menzel, 2001). In the mushroom bodies too, the input to Kenyon cells, measured with optical imaging (Wang et al., 2001), is significantly more widespread than the output of these (Laurent and Naraghi, 1994). Our results suggest that indeed, the dependence of olfactory bulb and antennal lobe response on concentration seen with optical imaging is not paralleled in the output of single neurons. A potential explanation of the source of the discrepancy between calcium imaging and electrophysiological studies is that because calcium is released by presynaptic terminals regardless of whether their effect is excitatory or inhibitory, calcium imaging studies are likely to present a rectified version of the input to the area under study. This would explain the fact that, whereas intracellular recordings show the concentration-dependent strengthening of both excitation and inhibition balancing each other out, optical imaging studies see simply a strengthening of the input.

Fifth, the balancing of excitation and inhibition we observe may well serve the purpose of

gain control, allowing the antennal lobe to reduce the dynamic range of its output in the face of large changes in its input.

Finally, the functional role of slow inhibition in the antennal lobe and olfactory bulb has been the subject of controversy (Laurent, 1997, 1999). It has been hypothesized that this inhibition is the result of lateral inhibition serving the purpose of increasing the difference of activity between the least and most active glomeruli for each odor to yield efficient coding and increase odor discriminability (Rospars and Fort, 1994; Yokoi et al., 1995). The role of lateral inhibition has been questioned recently, though (Laurent, 2000). The results presented here, showing the monotonous increase in both excitatory and inhibitory responses to increasing concentration, suggest a different and perhaps more fundamental role for inhibition in PN responses. If the presence of odors is decoded by KCs detecting a particular combination of active PNs, and if a blend of odors is *not* detected as the sum total of all subsets of odors in the mixture but rather as a new odor in and of itself (Laing and Francis, 1989) –the chemical signature of an event—, then an inhibitory mechanism is needed to prevent particular KCs from being activated in response to a subset of components occurring in a blend, ensuring that only the KCs that signal for the whole blend are activated (see Appendix II). When the output is which PN combinations are active simultaneously and which combinations are *not*, the role of inhibition in signaling is just as important as that of excitation.

Our results lead to the prediction that selectively blocking slow inhibition will elicit novel PN responses to blends containing a response-evoking odor, and lead to an increase of false positive behavioral responses to high concentrations and odor blends containing a component to which an animal has been trained to respond selectively.

Methods

Specimens, odor stimulation and electrophysiology

Intracellular recordings were obtained from 180 cell-odor pairs in 46 PNs of 21 locusts, *Schistocerca americana*. Surgery and recordings were performed as previously described (Laurent and Davidowitz, 1994; Laurent et al., 1996). Delivery of seven odors, including pure compounds as well as ethologically relevant blends, was performed using a gaseous dilution computerized odor-delivery system capable of delivering arbitrary concentrations by mixing a stream carrying saturated odor vapor with a second stream carrying pure air. The concentration of the odor delivered was regulated by controlling the relative flow rates of both streams (see Chapter 6). The system was purged between presentation of different stimuli. The stimulus sequence was delivered to an initially naïve animal, that is, one that had no prior experience with the odor tested. Successive stimuli presentations were spaced 10 seconds apart. No adaptation was observed between successive presentations.

The odor timecourse and magnitude was measured by using CO_2 as a tracer in the air line carrying the odor while the diluting stream carried ambient air, and measuring the CO_2 concentration at the nozzle (see Chapter 6). Direct measurement of the odorant concentration with Gas Chromatography Mass Spectroscopy also showed that concentration returned to baseline within 1 minute of purging.

Data were digitized at 20 kHz using a digital tape player (DAT, Biologic) and then acquired into a personal computer at 4 kHz using an analog/digital input/output card (National Instruments). Analysis was conducted on raw intracellular voltage traces as well as spike rasters. Spike isolation was conducted by voltage thresholding and visual inspection.

PN sensitivity to concentration

Each PN-odor pair was challenged with multiple trials (n>=5; n>=10 for most datasets) at each of at least two concentrations between 2 and 100% of saturated vapor pressure. The firing rates for different concentrations were compared using a paired T-test comparing maximum (over time) mean (over trials) firing rates for the highest and lowest concentration tested for each cell-odor pair during 4 sec following odor onset, for the 65 cell-odor pairs with only excitatory responses in the spike trains. A response was defined as excitatory if one or more epochs (300 or 500 msec, shifted in 100 msec steps, to account for short responses with high temporal precision as well as longer responses with more variance in spike times) exhibited a firing rate increase to at least 3 standard deviations above the mean baseline rate.

The low baseline firing rates of PNs (2-6 Hz) prevented an accurate evaluation of inhibition using firing frequency alone. Instead, the intracellular voltage traces for successive trials of the same concentrations were aligned on the mean voltage during the second preceding odor delivery for each trial. An odor was said to elicit an inhibitory response at a given concentration if there was any period (evaluated with sliding windows of 500 msec shifted in 100 msec steps) during which firing was suppressed and the mean voltage was at least 3 standard deviations below the mean voltage during the period preceding odor delivery. 35% of datasets showed some period of inhibition and individual responses often contained a period of excitation and a period of inhibition. The depth and duration of inhibition were quantified for each concentration. Depth was defined as the mean voltage during all inhibited epochs. Duration was defined as the total duration of all epochs during which inhibition was detected.

Peri-stimulus time histograms (PSTHs) were computed by calculating the mean firing rate for successive 100 ms bins.

LFP sensitivity to concentration

LFP traces were resampled to 1 kHz with MATLAB's resample function and filtered with a 13-30 Hz 5th order Butterworth non-causal bandpass filter. Filtered traces were processed to find local maxima and minima. Maxima (minima) were identified by locating zero-crossings that coincided with a positive (negative) second derivative. A cycle's peak-to-peak amplitude was defined as the absolute difference between the corresponding maximum and minimum. To ensure robustness of the effects observed, measurements were done both with all trials and with trials with the single greatest amplitude discarded as outliers for every concentration, with similar results.

Phase analysis

The phase of spikes was defined as

$$\text{phase} = \text{pi} * M + \text{pi} \,.\, f,$$

where f is the fraction of time elapsed between the previous FP extreme (maximum or minimum) and and the time of occurrence of the spike:

$$f = (t_{spike} - t_{previousextreme}) / (t_{nextextreme} - t_{previousextreme})$$

and M was 1 if the previous FP extreme was a minimum and 0 if it was a maximum. We also experimented defining phases with respect to every quarter cycle and every full cycle, but half cycles proved the most consistent across trials.

Naive vs. experienced animals

For every dataset, a trial was defined as naïve if the animal had not been exposed to the same odor at a higher concentration before, and post-high-concentration if the animal had been exposed to the same odor at a higher concentration before (see Chapter 9).