CHAPTER 7

Multiplexing odor identity and concentration information with a population temporal code

Abstract

The nervous system faces a dual task in the representation of sensory stimuli. On the one hand, exquisite sensitivity to differences in the stimulus requires different representations for each different stimulus. On the other hand, robust recognition of a stimulus under varied conditions requires invariance to changes in intensity, position, … In olfaction, psychophysical studies show that individual odors can be perceived as identical over significant ranges of concentrations (Gross-Isseroff and Lancet, 1988; Bhagavan and Smith, 1997). And yet some compounds are edible at low concentrations and toxic at high concentrations (McKechnie and Morgan, 1982; Van Delden 1982; Chakir et al., 1993), so the ability to discriminate concentrations has a selective advantage. How the brain deals with this trade-off is currently unknown. While odor identity information has long been thought to be encoded by the identity of the neurons responding and by slow temporal response patterns (Laurent and Davidowitz, 1994; Wehr and Laurent, 1996), how intensity information is multiplexed with the identity signal has remained controversial. One hypothesis proposes that odor identity and odor concentration information is multiplexed in the same neurons by having the phase of action potentials with respect to the local field potential code for concentration (Hopfield, 1995). Here, I record simultaneously from individual projection neurons in the antennal lobe of the locust and the local field potential from their target area, the mushroom body calyx, and find that the phase is constant across concentrations, contrary to the prediction of the model. A second hypothesis proposes that concentration information is carried in the response to initial exposure, but that the changes induced by short-term plasticity (Stopfer and Laurent, 1999) might make subsequent trials are less sensitive to concentration (Stopfer and Laurent, 2000). My data shows this not to be the case either: concentration information is contained in all trials (exposure to high concentrations, though, makes responses more similar across concentrations; see Chapter 9). A third hypothesis proposes that increasing concentrations introduce additional spikes that are not locked to the local

field potential (Stopfer and Laurent, 2000). This hypothesis has the attractive feature that the extra spikes could be filtered out by Kenyon cells selective for synchronized activity to achieve concentration invariance. My data, however, argues against this hypothesis too: PNs do not on average respond to higher concentrations with extra spikes, and even those that do show tighter synchronization with increasing concentrations, contrary to what one would predict. Based on my data, I present a fourth hypothesis, namely, that concentration is coded by the tightness of synchronization across PN assemblies.

Introduction

Behaviorally relevant concentrations range from detection threshold —for a dog following a faint track— to saturated vapor pressure very close to the source —inside a flower for a bee or against a potential mate's behind for a dog. Fruit flies feed and deposit eggs on fermenting plant materials in which ethanol is the most abundant short-chain alcohol (McKechnie and Morgan, 1982; Van Delden 1982). The ability to detect ethanol is thus important for chemotaxis toward food sources. However, adult flies are also susceptible to intoxication and death in high ethanol environments (Chakir et al., 1993). Therefore, there is a selective advantage for the ability to avoid environments with dangerously high alcohol concentrations. In Drosophila, for example, the LUSH gene has been determined to be required specifically for the response to high concentrations (Kim et al., 1998). This illustrates the selective advantage conferred by the ability to discriminate concentrations of an odor which have different behavioral relevance.

While large changes in odor concentration are known to change the identity of the neurons activated (Kauer, 1974), how the system encodes smaller changes in concentration, over which the

identity code must remain invariant to allow for perceptual recognition, remains unknown.

Results

A recent hypothesis proposes that odor concentration is encoded by the phase of action potentials with respect to an oscillatory drive (Hopfield, 1995). To test this hypothesis, I carried out intracellular recordings from PNs simultaneously with the local field potential (LFP) in the calyx of the ipsilateral mushroom body while delivering various concentrations of 7 odors. I computed the phase of each spike with respect to the LFP (see Methods). Contrary to the prediction of the Hopfield model, the phase remained constant not only for different odors (Fig. 7.1; see also Laurent and Davidowitz, 1994; Wehr and Laurent, 1996), but also for different concentrations of any one odor (phase for maximum concentration not significantly different from that at minimum concentration tested, $p>0.2$ both in naive animals and after exposure to higher concentrations, Wilcoxon rank-sum test, n=165 PN-odor pairs, see Fig. 7.2).

Contrary to a previous report (Laurent and Davidowitz, 1994), the peak phase at which PNs synchronized to the LFP varied significantly across recordings (compare Figs. 7.1, 7.2 and 7.5), and did not always occur during the rising phase of the LFP (see, for example, Fig. 7.5). Whether these differences were due to PN identity or to the position of the LFP electrode remains to be determined.

A second hypothesis put forth based on preliminary evidence (Stopfer and Laurent, 2000) is that information about concentration could be contained in the intensity of bursts upon initial exposure to an odor, but reduced by the effect of short-term plasticity on PN responses (Stopfer and Laurent, 1999). To test this hypothesis, I quantified the difference between responses to different concentrations for initial as well as later exposures to each of seven odors. Responses were signifi-

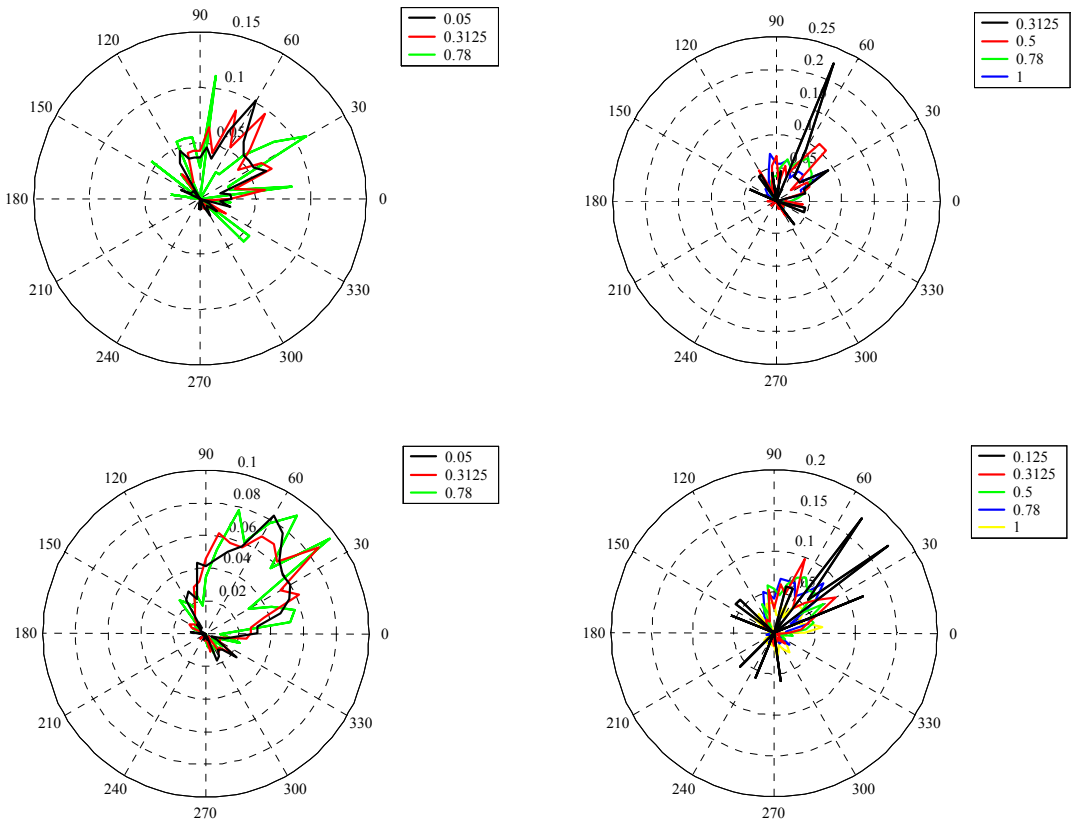

Figure 7.1. A PN synchronizes to the LFP at the same phase for different odors. From top left and clockwise, the same PN's response to cineole, citral, geraniol and octanol.

cantly different for different concentrations both for initial and later responses. In fact, trial number had no significant effect on the difference between responses to different concentrations, both for lower concentrations ($p>0.9999$, ANOVA, n=51 PN-odor pairs, see Fig. 7.3) and higher concentrations ($p>0.8935$, ANOVA, n=51 PN-odor pairs). Note that a different kind of plasticity induced by exposure to higher concentrations of the same odor, though, can indeed reduce the dependence of PN responses on concentration (see Chapter 9).

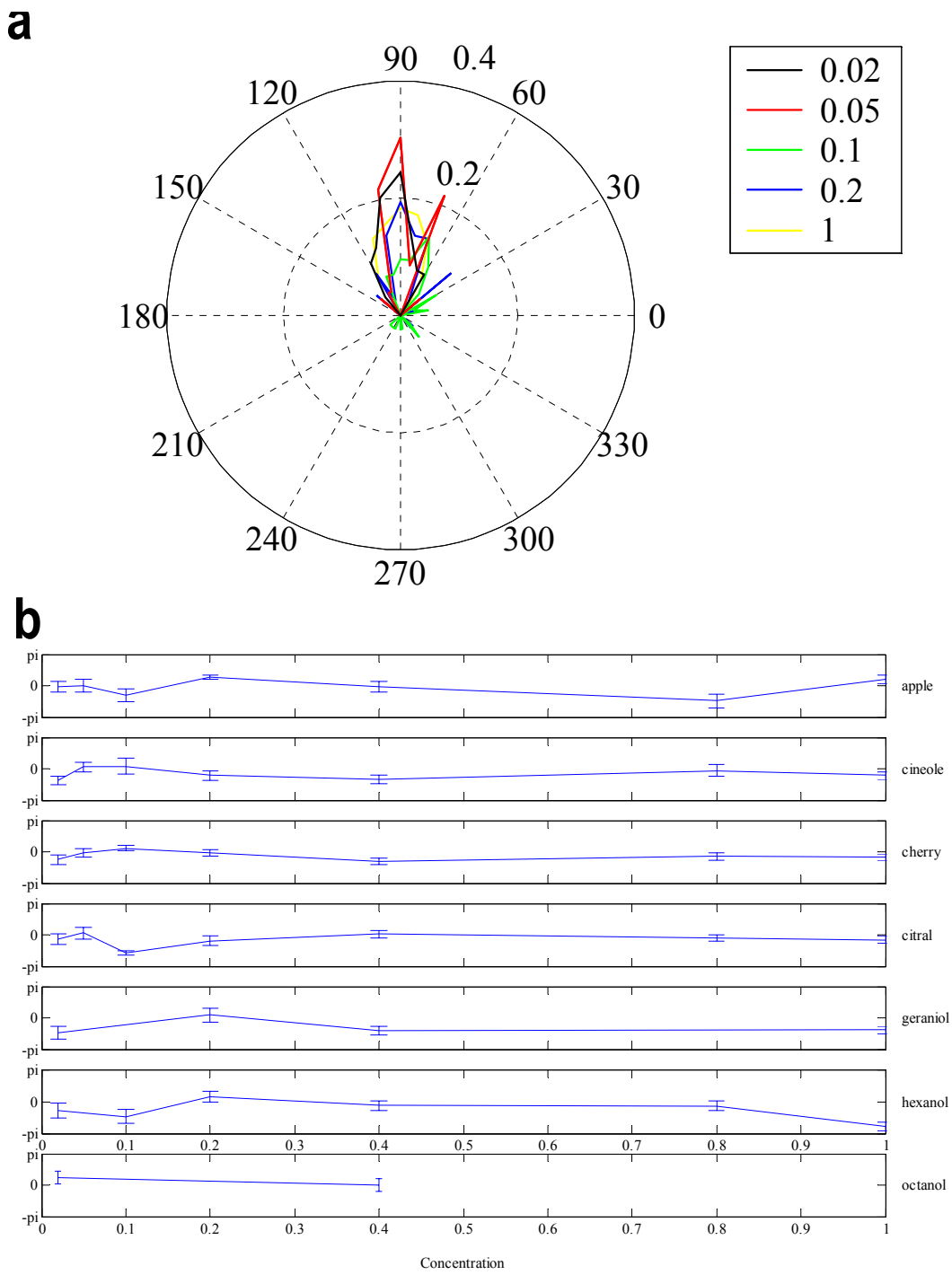

Figure 7.2. The phase of PN spikes with respect to the LFP remains constant across concentrations. a) Polar plot of a PN's spikes' phases in response to apple at various concentrations. **b)** Mean phase (and s.e.m.) for different concentrations of each of seven odors, averaged across 170 PN-odor pairs.

A third hypothesis suggested by preliminary evidence is that information about concentration is encoded in extra spikes that appear at higher concentrations in between the spikes that are locked to the FP (Stopfer and Laurent, 2000). This hypothesis has the attractive feature that a downstream neuron that was sensitive only to synchronized spikes would be invariant to concentration changes, while one which was sensitive to all spikes would be sensitive to concentration. This would enable the animal to retain information about concentration while achieving the invariance necessary for robust recognition. This hypothesis predicts that the degree of synchronization between PNs and the LFP will decrease with increasing concentration, as extra spikes ar**v**e elicited. My data, however, indicate that the opposite is true: the proportion of spikes that are synchronized to the LFP increases with increasing concentration (Figs. 7.4 and 7.6). Some PNs' (33% of the 71% that showed synchronization at any time) spikes were synchronized to the LFP at all times, during, before and after odor responses (Figs. 7.5 and 7.7). No such synchronization was present for randomly generated spike trains or uniform spike trains with a spike every millisecond. Synchronization could be measured even though the LFP's amplitude was signficantly smaller outside of the periods of odor responses. For these PNs, synchronization did not change with concentration. Other PNs (67% of those that showed synchronization at any time) synchronized only in response to odors. For these, synchronization increased with increasing concentration (Fig. 7.4 and 7.6). I never observed the synchronization of the spikes in an odor response decrease with increasing concentration (n=170 PN-odor pairs in 46 PNs). Over all PNs, the proportion of spikes between -pi/2 and pi/4, where 0 is defined as the peak of the LFP, was signficantly larger for the maximum concentration than for the minimum concentration tested ($p<3x10-4$, Wilcoxon ranksum test, n=170 PN-odor pairs in 46 PNs, Fig. 7.6).

If synchronization indeed codes for concentration, an important question arises: is the concentration sensitivity of synchronization eliminated by priming (see Chapter 9), or is it robust to

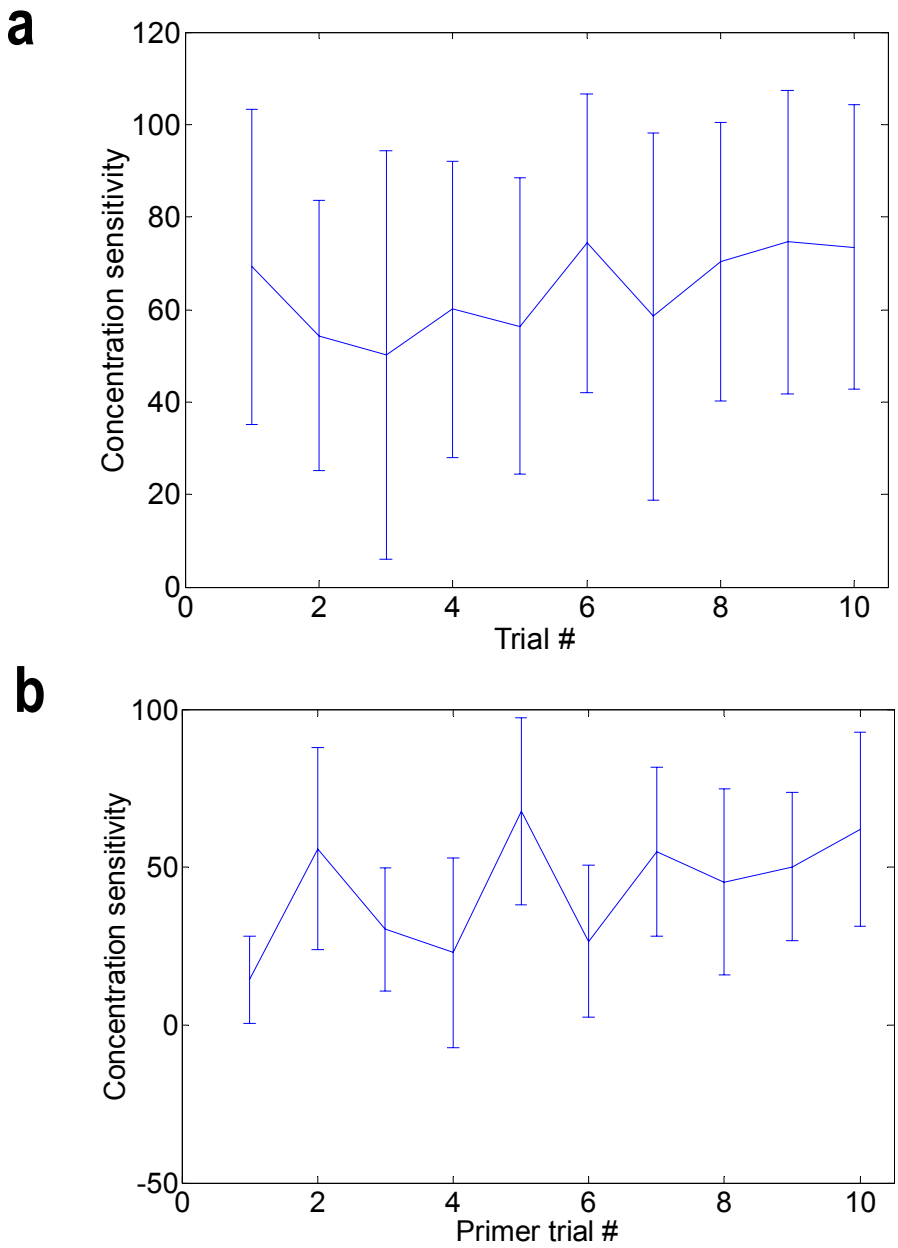

Figure 7.3. Concentration sensitivity is not affected by succesive exposures to the same odor stimulus. Concentration sensitivity (CS) for trial #N was defined as CS=Cross - Self, where Cross is the mean distance between trial #N at the **(a)** lower or **(b)** higher of two concentrations and all 10 trials in a series at the other of the two concentrations, and Self is the mean distance between trial #N at the **(a)** lower and **(b)** higher concentration and all the other trials for the same concentration. Plots show means over 55 PN-odor pairs and s.e.m. The series at low concentrations were presented before those at high concentrations since responses were affected by exposure to higher but not to lower concentrations (see Chapter 9).

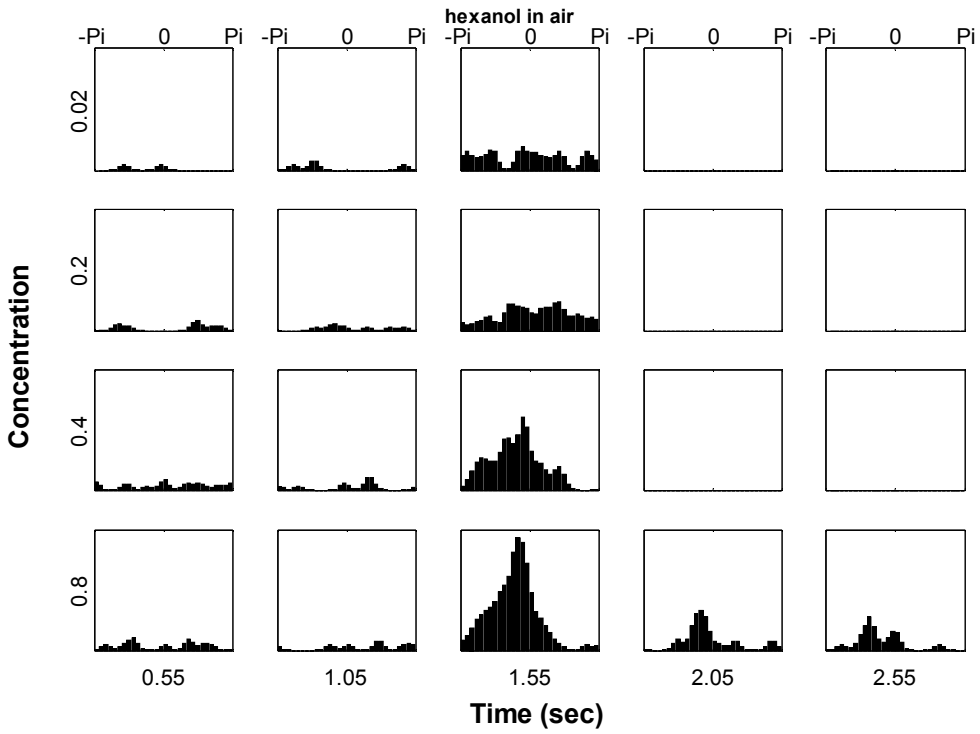

Figure 7.4. Synchronization of a PN's spikes to the LFP gets tighter with increasing concentration. Each plot shows a histogram of phases for a 500 ms window centered at the indicated time. The odor was presented between t=1s and t=2s. Notice only spikes during the odor response are synchronized, and then only at high concentrations.

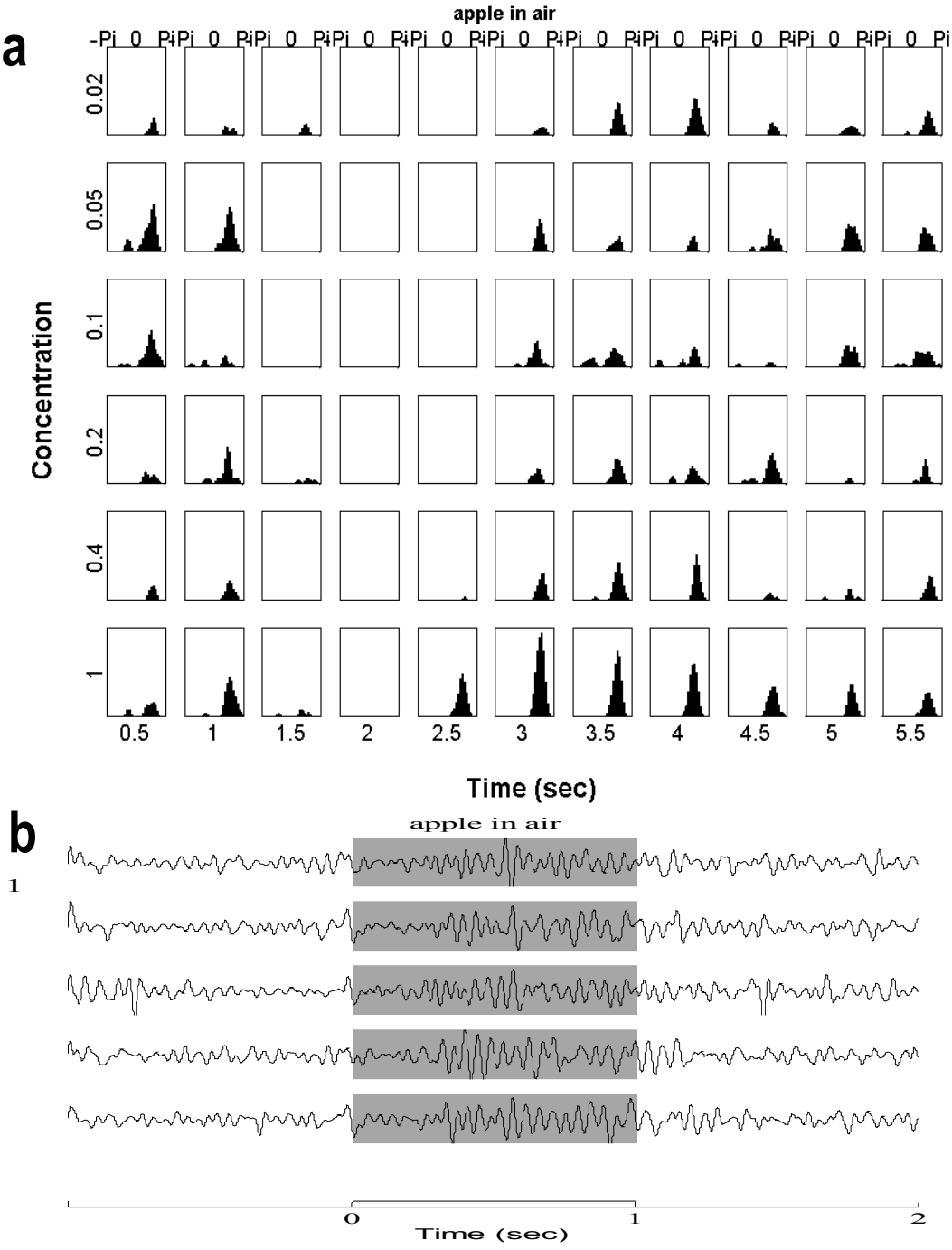

Figure 7.5. Some PNs are continually synchronized to the LFP. a) Synchronization does not vary with concentration. The phase is also constant throughout the recording and for all concentrations tested. Each plot shows a histogram of phases for a 500 ms window centered at the indicated time. The odor was presented between t=1s and t=2s. **b)** Filtered LFP traces for 5 of the trials of 100% concentration. Note that cycles are sometimes visible even in the absence of odor presentation.

exposure to high concentrations? Interestingly, I found that the effect of concentration on synchronization is robust to exposure to higher concentrations: contrary to the effect of priming on the concentration sensitivity of single neuron responses, the concentration sensitivity of synchronization is, if anything, enhanced by exposure to higher concentrations (Fig. 7.6b).

Conclusions

By multiplexing odor quality information in the slow temporal patterns with concentration information in the synchronization across neurons, the olfactory system might effectively solve the problem of achieving invariance to concentration while keeping concentration information as well. Furthermore, this dissociation allows the system to manipulate odor identity and concentration information separately, as priming does (see Chapter 9).

How would such an encoding scheme be decoded? The answer depends on whether the decoder's goal is to recognize odor identity, independent of concentration, or odor concentration. Animals are presumably interested in both (McKechnie and Morgan, 1982; Van Delden 1982; Chakir et al., 1993; Kim et al., 1998). Interestingly, PNs have two known distinct targets: Kenyon cells in the mushroom body, and lateral horn interneurons (LHIs). Kenyon cells (KCs) are odor selective and fire only during part of each LFP cycle (Laurent and Naraghi, 1994; Perez-Orive et al., submitted). Furthermore, KCs appear to exhibit a prolonged hyperpolarization after every spike, and their odor responses are very sparse, often responding with a single spike in any one oscillation cycle, or even throughout an entire odor response (Laurent and Naraghi, 1994; Perez-Orive et al, submitted). LHIs, on the other hand, respond vigorously to odor stimulation and show very limited odor-selectivity. The LHI population fires spikes during over half of each cycle, and sends inhibitory projections to

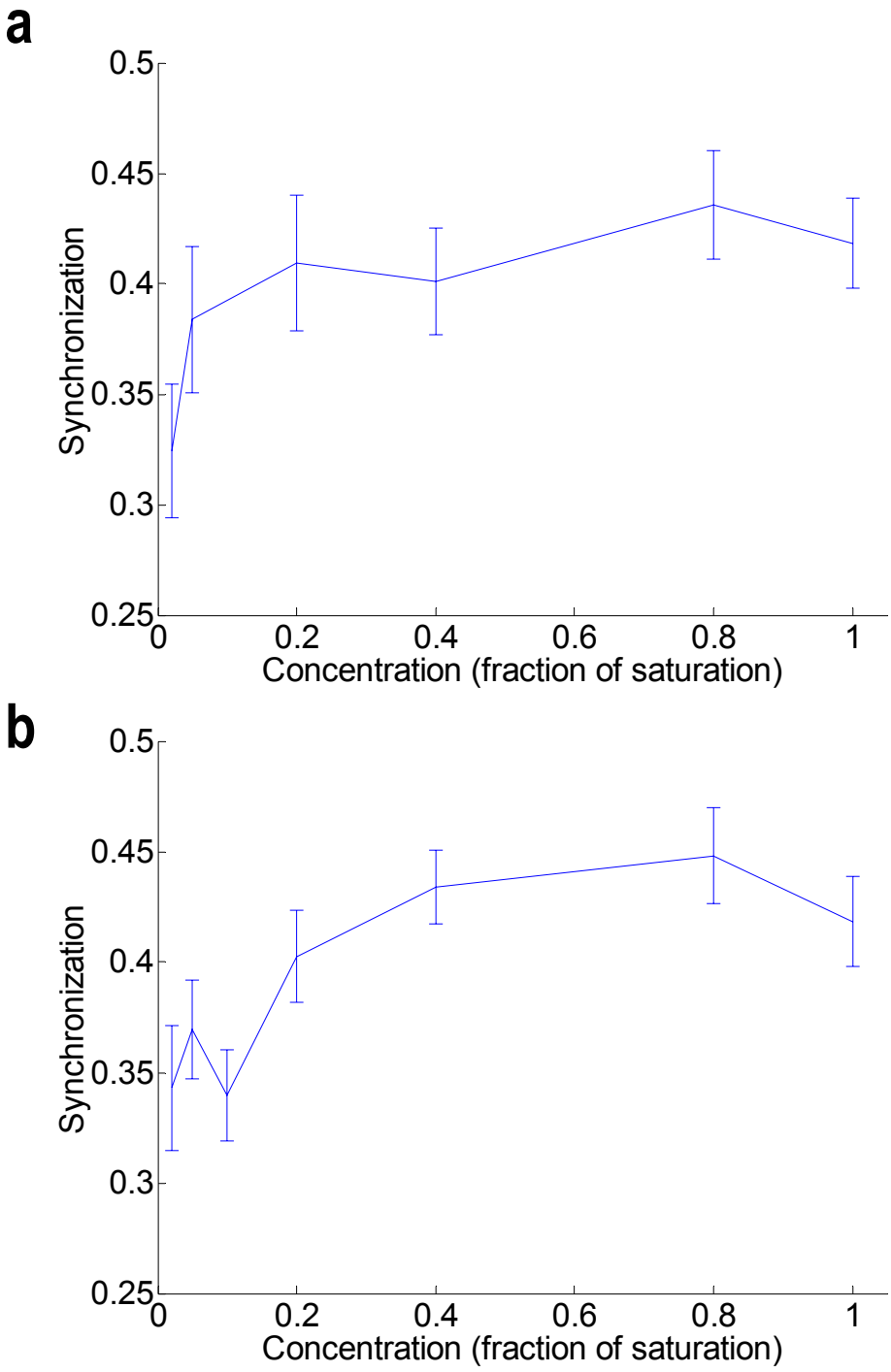

Figure 7.6. Synchronization between PNs and the LFP increases as a function of concentration, both in naive animals (a) and after exposure to higher concentrations (b). Mean fraction of spikes that fall between pi/2 before LFP peaks and pi/4 after them, averaged over 170 (a) and 165 (b) PN-odor pairs, and s.e.m.

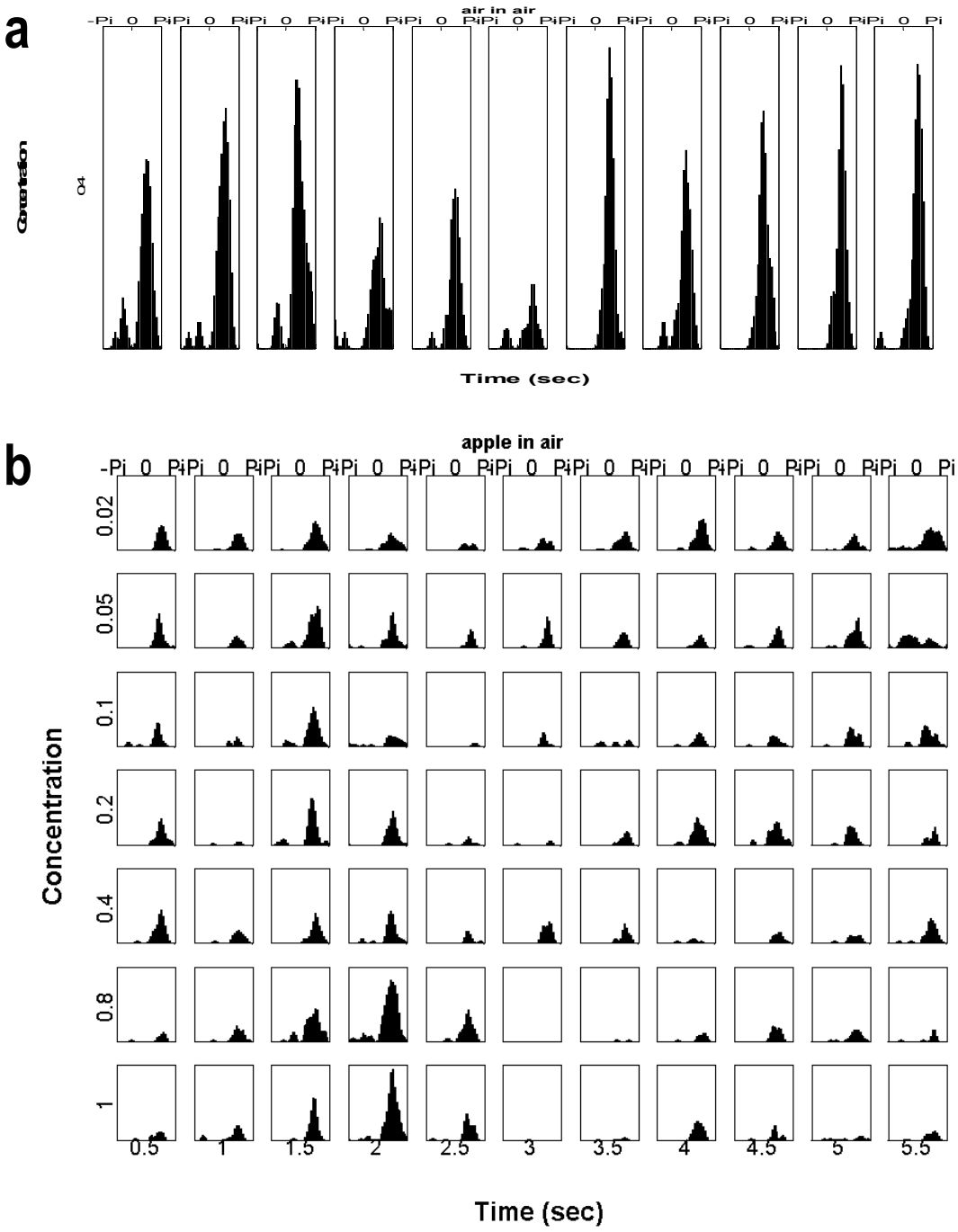

Figure 7.7. A different PN from that in Fig. 7.5 is continually synchronized to the LFP during baseline firing and responses to air (a) and apple (b). Synchronization does not vary with concentration. The phase is also constant throughout the recording and for all concentrations tested. Each plot shows a histogram of phases for a 500 ms window centered at the indicated time. The odor was presented between t=1s and t=2s.

the mushroom body. It is tempting (though premature) to speculate that Kenyon cells might respond with a single spike whenever the corresponding set of PNs fires synchronously enough to exceed threshold, and thus be relatively invariant to suprathreshold concentrations, since one a KC's threshold for firing is reached, further synchronization would not make them fire more than one spike. LHI's, on the other hand, might respond more vigorously to a volley of PN input if it is more synchronized, as it would be for higher concentrations. The ensuing stronger inhibition feeding back on KCs might itself act as a mechanism of gain control, keeping the output of KCs invariant to concentration. Preliminary recordings from LHIs suggests that LHIs indeed respond more robustly, and in a more periodically synchronized way, to higher concentrations (Glenn Turner, personal communication).

A caveat to note is that it is conceivable that the higher degree of synchronization observed is due to the higher amplitude of the local field potential oscillations at higher concentrations, which elevates the signal-to-noise ratio and might make synchronization measurements less noisy. To test this possibility, I computed the spike-triggered average of the spike trains for each concentration across all odors and the 46 cells, to test whether any periodicity became more pronounced and/or more tightly locked as concentration was increased. No significant periodicity was observed at any concentration. I then computed the autocorrelation of the intracellular membrane voltage for all odor responses as a function of concentration. Once again, although periodicity was observed in some individual datasets, no periodicity was observed in the averages across PNs, possibly because the frequency of the oscillatory drive to PNs might change slightly across different cells or possibly even from trial to trial. A potential test to control for the LFP amplitude being responsible for the observed increase of synchronization with concentration would be to record from multiple PNs simultaneously and measure the number of spike coincidences as a function of concentration. It is unlikely that the FP amplitude can account for the differences observed in synchronization, however, since tight synchronization was clearly observable even at the very low LFP amplitudes present in the absence of

odor stimulation for PNs which exhibited permanent synchronization (see, for example, Fig. 7.5).

The demonstration that PNs are synchronized to the LFP over a range of concentrations spanning two orders of magnitude suggests that this coding scheme is not restricted to high concentrations (see also Chapter 8).

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 GCMS 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.

Phase analysis

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. 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. I 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 naive 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).