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Time-varying phase relationship between spiking neuronal responses and local field potentials in olfactory processing in *manduca sexta*

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Time-Varying Phase Relationship between
Spiking Neuronal Responses and Local Field
Potentials in Olfactory Processing
in *Manduca sexta*

Oakland J. Peters

Thesis submitted to the
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Abstract

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Oakland J. Peters

The Transient Oscillatory Model (TOM) seeks to explain neural coding in the first processing center of the invertebrate olfactory system – the antennal lobe – by proposing that activated primary output neurons participate in the encoding ensemble only transiently, based on the coincidence of their spiking relative to local field potential (LFP) oscillation phase. Using the sphinx moth, *Manduca sexta*, as a model system, we tested the predictions of the TOM with regard to the trial-to-trial consistency of odor driven oscillations, the phase-locking of action potentials to LFP oscillations, and the role of GABA_A receptors in the generation of these oscillations. We quantified the changes in LFP oscillation frequency over time via time-frequency representation (TFR) analysis, and calculated the timing of action potentials relative to the phase of the LFP oscillations (vector-strength analysis). In accordance to the TOM's predictions for the role of LFP oscillations, TFRs revealed that odor-driven oscillations modulate in a stimulus specific manner. However, contrasting with these same predictions, vector-strength analyses illustrated that phase locking was higher during spontaneous activity than during odor response. Finally, in disagreement with TOM predictions, disruption of GABA_A receptors by BMI application reduced odor-driven LFP oscillations across time-periods and regions of the AL. Further BMI application reduced phase-locking of action potentials to the LFP, while leaving phase-locking highest during spontaneous activity. Consequently, we find the overall architecture of the TOM to be incompatible with these findings.

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Dedication

I dedicate this thesis to my family – Kay & James Hinzman, Goodman Griffin and Ann Renner-Griffin, and Rax Elsir, for their love and support which has made this possible.

Introduction

Olfactory stimulation gives rise to bursts of neural activity in the first olfactory processing center, the antennal lobe (AL) in insects, or the homologous structure in vertebrates, the olfactory bulb (OB). In the AL, this odor driven response is vividly observable in the patterns of spiking activity of the primary outputs, the projection neurons (PNs). Diffuse subthreshold electrophysiological oscillations in the extracellular matrix, called local field potential oscillations (LFPOs), are correlated with and potentially drive PN activity (Laurent and Davidowitz, 1994). LFPOs represent aggregate reciprocal synaptic activity from a restricted region. Indeed, there is considerable evidence that LFPOs reflect the synchronization of neural assemblies (Laurent, 2002; Laurent et al., 1996a). In the mammalian cortex, modulation of LFP phase and power correlates with spiking activity (Rasch et al., 2008).

Physiological context of the AL

The olfactory pathway begins at odor signal transduction, when 7-transmembrane spanning, G-protein-coupled receptors located on olfactory receptor neurons (ORNs) bind odorant molecules (Vosshall et al., 1999). In many species (including *Manduca sexta*), each ORN expresses genes for one or at most a few receptor protein types (Vosshall et al., 1999). Each receptor protein has a chemical affinity for a restricted range of odorants, and the affinities for subsets of proteins can overlap. Consequently, even very pure odorants cause the activation of multiple ORN types.

After signal transduction, ORNs generate and transmit action potentials along their axons, into the AL. Within the AL, individual ORNs project to one of many dense spherical regions of neuropil called glomeruli, which are the primary organizational unit of both the AL and OB, and all ORNs expressing the same receptor profile project to the same glomerulus (Mombaerts et al., 1996; Vosshall et al., 1999).

Glomeruli in insects are generally individually identifiable and modest in number. For example in *Drosophila melanogaster* there are more than 40 olfactory glomeruli while in *Manduca* there are ~64 plus the male-specific macroglomerular complex. Conversely, in mammals glomeruli are rarely individually identifiable, and much greater in number – for example mice have around 1800, and humans estimates are between 1000 (Pinching and Powell, 1971) and more than 5000 (Rodriguez-Gil et al., 2013). The locust presents a notable counter-example of this trend in insects, having approximately 1,000 microglomeruli which are generally not individually identifiable (Laurent et al., 2001).

The primary AL output neurons, projection neurons (PNs), have glomerular dendritic arborizations, and send axons to higher processing centers. In insects, this includes the mushroom bodies (MB) and lateral horn (LH) – centers of memory and behavior. Local interneurons (LNs) span multiple glomeruli (sometimes all), receive direct ORN synaptic input, and are known to form reciprocal connections with PNs. In *Manduca sexta*, they are primarily GABAergic and hence inhibitory (Christensen et al., 1993).

Function of Glomerular Networks

As mentioned, individual glomeruli receive projections from ORNs expressing either a single olfactory receptor protein, or at most a small number of receptor types. This is likely a common feature of olfactory systems generally.

Therefore, it was not uncommon in early olfactory coding theories to describe patterns of glomerular activation (even after being subject to LN inhibition) as a reasonably direct proxy for activation of ORN types. This understanding could be described as a relatively coarse glomerular spatial encoding scheme (Wilson and Leon, 1988). However, this understanding has since evolved into more nuanced spatial encoding theories. These include spatio-temporal coding theories (Daly et al., 2004a; Laurent et al., 1996b; Wilson et al., 2004), which propose that the initial pattern of input is somehow transformed through lateral interactions established in the local network. Spatio-temporal theories have been bolstered by the discovery of excitatory lateral connections within the AL (Wilson et al., 2004). These excitatory connections are associated with cholinergic LNs which respond to a very broad range of ORNs and project very widely across glomeruli (Shang et al., 2007). Consequently, the tuning profile of many PNs (and the glomeruli they contribute to) is considerably broader than the profile of their presynaptic ORNs.

Further, it has been shown that AL responses can change as a function of olfactory experience, which implicates learning (Daly et al., 2004a; Stopfer and Laurent, 1999). This implies that the AL is an active locus for olfactory learning. Therefore glomerular activation levels must necessarily represent more than simply a static combinatorial encoding of ORN activation. Thus odor identity encoding is a more complicated and dynamic transformation than was previously envisaged. A significant part of this broader picture of dynamic AL activity relates to inhibitory GABAergic LNs and their reciprocal interactions with PNs, and the relationship between this reciprocal activity and the production of LFPOs.

Function of LN Networks

Traditional theories of odor encoding propose exclusive functions for LN networks. For example the canonical spatial encoding theory predicts that LN function is exclusively for contrast enhancement (Sachse and Galizia, 2002). However, excitatory LN activity may act to amplify weak ORN inputs relative to strong ones (Bhandawat et al., 2007), which has the function of increasing the 'dynamic range' of PN tuning in regard to odorant concentration.

On the other hand, LN-PN reciprocal connections are hypothesized to heighten synchronization between PNs ramifying glomeruli, which are distributed across the AL/OB (Bazhenov et al., 2001). This increase in synchronization would arise via the following mechanism. PN activity excites afferent GABAergic inhibitory LNs. These LNs then inhibit both the pre-synaptic PN (a reciprocal dendro-dendritic synaptic configuration), and related PNs. As this inhibition decays, PN activity will resume across the PNs previously inhibited, again invoking recurrent inhibition. The result is cyclic periods of synchronized PN bursting, with the precision and frequency of bursts being dependent on the properties of GABAergic LN inhibition (Desmaisons et al., 1999; Lagier et al., 2004).

Utility of LFPOs

Spiking activity of OB output cells (mitral cells; analogs of PNs) arises contemporaneously to LFP oscillations, and cross-correlation of this spiking activity shows peaks corresponding to the dominant LFP frequency (Schoppa, 2006). Further, it has been shown that spikes which are coincident tend to be more locked (or correlated) to LFP oscillations than would be those spikes separately (Denker et al., 2011).

Consequently, LFPOs may at least be used as a valuable proxy indicator for the synchronization of PN ensembles. Further, there is activity, which is unrelated to stimulation, instead reflecting ongoing spontaneous activity (or 'network state'); this activity is not reflective of sensory encoding (Kelly et al., 2010). Thus, LFP captures population or network level activity not otherwise available. Hence, LFPOs may be used to estimate fluctuations in activity across entire networks, which would otherwise be difficult to measure with samples drawn at the level of individual neurons.

While LFPO frequency spectral content for a given neuropil, such as the AL or OB, tends to be consistent within a species, this content varies greatly across species. LFPO frequency was found to vary with stimulus intensity in the visual cortex of the cat (Gray and Di Prisco, 1997) but unaffected by odor concentration in the locust olfactory system (Assisi et al., 2007; Stopfer et al., 2003) or *Drosophila* (Ito et al., 2009). In contrast, LFPO frequency in *Manduca* has been found to be dependent on both odor and stimulus duration (Daly et al., 2011).

GABA-A Blockade

As many LNs are GABAergic, this provides a means for testing their function. Both picrotoxin (PTX), a noncompetitive GABA-A antagonist, and bicuculline methiodide (BMI), a competitive antagonist, have been used to study selective inhibition of PNs by the inhibitory LN network. By blocking the reciprocal relationship between LNs and PNs, GABA-A blockade leads to a reduction in odor-driven LFPOs (Laurent et al., 1996a), and it has been claimed that this has the functional consequence of reducing fine odor discrimination (Stopfer et al., 1997). Thus the interpretation of these studies has been that temporal coding, established by successive cycles of the LFPO establish the identity code for odor. However, GABA-A also disrupts the spatial constraint of the odor representation within the AL (Sachse and Galizia, 2002). Thus GABA blockade not only disrupts the temporal aspects of a response, it also disrupts the spatial aspects through loss of contrast enhancement established via lateral inhibition, which is observed in other sensory systems.

The Transient Oscillatory Model

This fast GABAergic LN inhibition can act to shape the fine timing of PN ensembles, often in a way which increases PN-PN synchrony. This has been observed in a wide range of species including the rat (Schoppa, 2006), the moth (Daly et al., 2011), and the locust (Laurent et al., 1996a). This observation plays a crucial role in a number of prominent theories of olfactory coding which purport that a significant factor in the meaning of PN activity down-stream lies in the time coincidence of PN ensemble subpopulations (MacLeod et al., 1998; Rabinovich et al., 2001). In this model, it is the *coincidence of spiking relative to LFP oscillation phase* which

determines membership in an encoding ensemble, thereby making the phase itself a coding mechanism (Wehr and Laurent, 1996). Thus, activated PNs in this theory may be said to participate in encoding ensembles only *transiently* since as individuals their responses only last for a portion of the entire response. A schematic of this model is provided in Figure 1.

Transient synchronization in an olfactory context was initially identified via analysis on pairs AL neurons (Laurent and Davidowitz, 1994). It was found that a subset of PNs and LNs showed rhythmic activity synchronized (or 'phase-locked') to stable 20 Hz LFPOs. Non-synchronized neurons could be quiescent, inhibited, or active but unsynchronized. Whether an individual neuron was synchronized or not would vary transiently over the time-course of the response. However, it was observed that the epoch of synchronization for individual neurons was consistent on repetitions of individual odorants. Thus, the authors hypothesized that the encoding of odor identity may lie in a temporal code – defined by a transiently evolving ensemble of synchronously oscillating AL neurons and hence the spatial component is dependent on time; this is commonly referred to as the *Transient Oscillatory Model* (TOM).

The TOM was originally developed in the context of the locust olfactory bulb, but its ideas of transient oscillatory synchronization have gained prominence, and subsequently been explored in a range of other systems. This model of neural encoding has since seen application in the olfactory bulb (OB) of the rabbit (Kashiwadani et al., 1999), the zebrafish (Friedrich et al., 2004), and the rat (Schoppa, 2006).

Dynamic Systems Formulation

Several TOM theorists have cast this model of an evolving spatiotemporal ensemble code into the language of dynamical systems, which describes the entire AL system at a moment in time as a single point in a space defined by the intensity of spiking of each PN. Each PN in the AL is represented as one dimension or axis in the space. As PNs enter or leave the responding ensemble, the point representing the activity level of the AL will follow a trajectory through that space (Laurent, 2002). The TOM asserts that this trajectory is reliable from trial-to-trial, and *the trajectory elicited in response to similar but behaviorally distinguishable odorants will diverge over the lifetime of the response and do so on a time scale defined by the LFPO frequency*. In the language of dynamical systems, the trajectories decorrelate in the later epochs of stimulus response. In terms of perception and behavior - decorrelation of the representation of similar odors causes these representations to become less similar over time and thus distinguishable by the animal. Early in olfactory responses, similar odors cause similar spatiotemporal patterns of PN ensembles. As odor response progresses, these responses become decorrelated, and therefore more accurately reflect behaviorally-salient differences in odor identity.

TOM Predictions

LFPOs for a particular odor stimulus are distributed, consistent and stable in timing, duration, and frequency.

In order for LFPOs to synchronize PN spike timing from across the AL, it has to be distributed. This suggests that the frequency profile of a response should be common across LFP recording

sites. The TOM also predicts that odor stimulation will lead to LFPOs which are consistent in onset latency and duration, and which occur within a reasonably narrow and stable frequency range during the life-time of the olfactory response (Laurent et al., 1996b). Further, the TOM predicts that LFPO frequency should be independent of odor identity and the time-dynamics of the stimuli (Laurent and Davidowitz, 1994; Laurent et al., 1996b). This is attributed to the notion that it is the intrinsic circuitry that produces the oscillation, and not the details of the stimuli. LFPOs must be stable and constrained in frequency because they are presumed to be a timing reference for cycle-by-cycle PN synchronization. Furthermore, LFPOs in this model require a stable frequency throughout the life of the response. This is because the mushroom bodies are believed to integrate PN input which is spatially distributed across the AL. Here integration occurs inside a window of time imposed by delayed inhibitory MB control originating from the lateral horn (Laurent, 2002; Perez-Orive et al., 2002). As shown in Figure 2, output from the AL projects to both the AL and the LH. In LH, the TOM argues, excitatory input via the AL drives inhibitory LH output to the MB thereby closing the integration window.

Studies have shown that PN firing patterns are shaped by both the chemical identity and temporal-dynamics of olfactory stimuli (Christensen et al., 1998). This is a particularly important consideration for flying insects, where wing beating and plume tracking behaviors generate an olfactory context which is especially variable and intermittent from moment-to-moment (Tripathy et al., 2010). This consideration has been bolstered by recent findings that odor identity has a complicated interaction with plume tracking dynamics, beyond that which is attributable to ORN reception itself (Krishnan et al., 2011). Therefore, by proposing that LFPOs act as a timing reference, TOM theories have difficulty accounting for LFPOs which vary significantly in frequency and timing as a function of stimulus dynamics.

Stimulation drives heightened phase-locking.

The TOM predicts that the identity of active PNs accounts for coarse scale odor identity. This is a combinatorial spatial encoding schema, which attempts to account for the ability to distinguish relatively dissimilar odors. However, in this theory, the olfactory system distinguishes between similar odorants on the basis of fine timing differences – in the form of transient periods of PN-synchrony determined relative to LFPOs (Bazhenov et al., 2001).

More specifically, the TOM predicts that PN spiking activity should be ongoing and phase-locked even outside of olfactory stimulation (Wehr and Laurent, 1996). However, odor encoding should drive more intense bouts of synchronized phase-locking of PN ensembles. Therefore, if the TOM holds, we should expect phase-locking during spontaneous non-stimulus driven activity to be higher than expected by chance, and the aggregate level of PN phase-locking to be highest during an odor driven response.

GABA-A blockade selectively disrupts synchronization dynamics.

In the TOM, transient oscillatory synchronization arises from the action of fast GABAergic inhibition at the synaptic junction between LN and PNs. Further, because LFPOs in the AL are driven by the LN-PN reciprocal interactions, GABA-A mediates distributed oscillatory activity inside the AL. Consequently, the TOM predicts that GABA-A blockade should selectively eliminate odor-driven LFPOs, while abolishing odor-driven PN synchronization.

Experimental Overview

Our goal in the present study was to test several crucial TOM predictions, through tandem extracellular electrophysiological recording of LFP and spiking units, along with pharmacological manipulation of GABA receptor function. First, to test whether LFPO frequency was stable and reliable, we recorded LFPOs generated in response to repeated stimulation across a panel of odorants and stimulus durations. LFPOs were found to modulate in a stimulus specific manner. Downward modulating frequency over the duration of stimulation was found in almost all cases. Next, to test whether PN phase-locking was ongoing, we simultaneously recorded LFPOs and PN spiking units before, during and after olfactory stimulation. By calculating the phase of each spike relative to the LFPO, our measures of population-level phase locking indicated that significant levels of phase-locking do occur in ongoing non-stimulus driven activity. However in disagreement with the TOM, aggregate levels of phase-locking drop significantly in response to a stimulus. Finally, to assess the effect of GABA-blockade on LN-mediated lateral inhibition and consequent PN synchronization, we repeated our PN & LFPO measurements before, during, and after GABA-A blockade via BMI and PCT application. We found that GABA-A blockade reduces or eliminates odor-driven frequency modulating LFPOs. Blockade also decreased phase locking in spontaneous and odor-driven responses, yet phase locking remained higher during spontaneous activity.

Methods

Surgical Preparation

Our experimental methods have been previously detailed (Daly et al., 2004a, 2004b), although the computational methods implemented in this study are distinct. In brief, male *Manduca sexta* moths were reared on-site and allowed to age to 5-7 days after eclosion before surgical preparation. An example extracellular multielectrode physiological preparation is shown in Figure 2. The body of an intact male *Manduca sexta* moth was secured in a metal tube with the head exposed. A segment of the head capsule was removed and both right and left pharyngeal dilator muscles were carefully moved forward, exposing the brain, while leaving the antennal nerve intact. The antennae were then secured in place to prevent further movement. Both connective tissue and trachea were removed from the surface of the deutocerebrum (AL) and protocerebrum (MB and LH). Finally, a 16 channel silicon microprobe was inserted into the exposed AL using a micromanipulator. The output of the 16-channel (4 tetrodes) electrode array was fed through a 24 channel Neuralynx amplifier array. Four LFP recordings were made from one electrode from each tetraode and were sampled at 10,752 Hz, and spikes from all 16 channels were sampled at 32,000 Hz. On-board hardware applied a band-pass filter to the recordings, retraining 1-125 Hz components. We later applied a software windowed-sinc notch filter in order to remove 60 Hz line noise (+/- 2 Hz) and a 10 Hz high pass filter to remove low frequency content.

Olfactory Stimulation Paradigm

The surgical preparation does not damage the olfactory reception and processing centers, allowing olfactory responses to be tested via odorant delivered onto the antenna by means of a computer controlled shunt connected to a 3-way valve. First, charcoal-filtered clean air is passed through the 3-way valve until electronically activated, at which point air-flow was directed through a prepared odorant cartridge and onto the antenna (see Figure 3). After passing across the antenna, odorant laden air was drawn into an exhaust vent directly behind the surgical preparation.

A randomized panel consisting of nine pure monomolecular odors was presented to the antenna. Each presentation consisted of 20 pulses, 100 ms in duration, with 20 seconds of clean air between each pulse. We presented this odorant panel three times – before, during, and after washing a bath applied pharmacological treatment of the GABA-A receptor blocker BMI. We chose a dosage of 200 μ M based on prior pharmacological evidence of its effectiveness at this dilution in this model system (Christensen et al. 1998, Waldrop et al. 1987).

Spike sorting

Spike-sorting was performed using the program BubbleClust in MATLAB, provided by Neuralynx. Bubbleclust uses a k-th nearest neighbor algorithm in an abstract 12 dimensional wave form feature space, where each dimension corresponds to a feature calculated from the wave-form of a single action potential (such as total energy or peak amplitude) from one of the

four electrodes in the tetrode. Sorting efficiency was assessed using a variety of measures including ISI histograms, and measures of cluster separation.

LFP Filtering

We also employed another extension of the basic Fourier transform: frequency filtration techniques. We employed two filters. First, a 58-62 Hz windowed-sinc notch filter was applied in order to remove line noise. Some of our analyses involved averaging across sets of time-frequency representations (TFRs; discussed later), and since line-noise is highly consistent, it could easily be differentiated from biological signals. Second, a high-pass windowed-sinc filter was applied in order to remove frequency content below 10 Hz. This component of the signal was much higher power than the higher frequency content that this study is focused on. This low frequency content consisted mainly of a simple and consistent waveform shape that likely corresponds to the broad input into the AL from the antennal receptor cells.

Time-Frequency Representations

We calculated time-frequency representations of recorded LFPOs during all stimulations, to determine how LFPO frequency spectrum varies over the lifetime of olfactory response. To implement our TFR analyses, we chose to use the *short time Fourier transform* (STFT), a class of TFRs which include the spectrogram (the sub-variety eventually chosen) and the Gabor transform (also tested). The STFT was chosen because it possesses no cross-term (signal artifacts) and uses low amounts of computational resources. While the STFT has poor frequency resolution compared to other methods, this comparatively low level of resolution remains amply sufficient because the frequency width ('bandwidth') of processes visible in the LFP (< 150 Hz) is very wide compared to the sampling rate of modern day electrophysiology equipment (typically > 10,000 Hz).

Our spectrograms were implemented via the Time-Frequency Toolbox for MATLAB, which is provided freely by the Centre National de la Recherche Scientifique (<http://tftb.nongnu.org>). To highlight consistent aspects of oscillations while suppressing trial-to-trial variability in oscillatory activity, we averaged responses to odor stimulation across 20 repetitions of the same odor (as shown in Figure 4); this process was repeated for data collected during BMI treatment. Analyses were always performed within the same subject and recording site. By suppressing trial-to-trial variability, we were able to emphasize oscillations that were present but not necessarily phase aligned across trials. Further, we z-score normalized TFRs to facilitate direct comparison of frequency and power between recording sites and pharmacological treatments (pre- and post-BMI treatment).

Vector Strengths

Next we were interested in understanding the relationship between the oscillatory field activity of the AL and spiking behavior from cells intrinsic to the AL. We therefore calculated the phase the

LFPO at the time of each recorded spike (a schematic is provided in Figure 5), and statistically examined this population for evidence of a categorical relationship. More specifically, we calculated the complex (or analytic) form of the time series via the Hilbert transform. At the time of occurrence of a recorded spike, the instantaneous angle of this complex time series yields the spike's phase.

We then calculated the first and second statistical moments (i.e. the mean and variance) for individual spikes from each neural unit within the recorded sub-population. Because phase is distributed on a circle, which represents the cycling LFPO, these calculations had to be altered from their conventional (or 'linear') form to circular (or directional) statistics. The 'vector strength' is one method of more accurately presenting angular statistical data. A vector strength is a polar plot which represents a population of angles (in this case the LFP phases for each action potential in the spike train) by a line centered on the origin. The angle of that vector corresponds to the first circular moment of the population (the adjusted mean) while the length, or strength, of the vector represents the inverse of the variance of that population. Thus the vector 'points' in the mean direction for the population, and the longer the vector, the more similar the members. The vector strength value ranges from an upper limit of 1, for a population with no variance, to 0 for a population with infinite variance (i.e. evenly distributed on a circle).

For this vector strength analysis, we sampled spiking and LFP activity into three epochs. The first was 'spontaneous' activity which occurred prior to the stimulation itself (-300 ms to 0 ms before the stimulation). The second epoch was activity occurring during the 'early' period of the odor-driven response (50 ms to 350 ms after odor stimulation). And the third 'late' phase activity, was 350 ms to 650 ms after odor stimulation.

We chose the 'early' phase of odor-driven response to occur after the initial 0-50 ms period, to account for the time lag between the onset of the stimulus and the first evidence of a physiological response. Hence we chose the 'early' phase to capture this while ignoring potentially spontaneous activity in the 0-50 ms period.

The vector strength attempts to determine the degree of phase relationship between a given spike train and the LFPOs. The vector strength is more appropriate in cases of relatively pure sinusoidal input (for example Wallace, 2002). When multiple wave components (a range of frequency components), or the frequency of the wave components change (non-stationarity), this measure becomes less appropriate. This is one of the motivations for dividing the stimulus response epoch into early and late response epochs – so that frequency (which was generally undergoing notable modulation during the early epoch) did not change too much within a single analyzed population. In addition we also bandpass filtered (using the above methods) to two narrower frequency ranges (25-55Hz and 55-85Hz) and vector strength analysis was implemented using both.

Caution should be exercised when interpreting the mean spike phase (the angle of the vector). The mean phase is highly sensitive to nuisance parameters (i.e. it is not a robust statistic) – such as non-stationarity in the LFP, the position of an electrode, etc.

Therefore, we will generally employ more robust statistics, such as the 'vector length', which measures the variance in spike phases. This measure is considerably more stable, assuming a relatively modest number of spikes. As this measures how concentrated, or 'dispersed', a population of spikes is relative to a particular phase of an oscillation, a high vector length (~low variance; ~near 1) implies something fairly close to what might be envisioned as phase-locked spiking activity.

Results

The transient oscillatory model predicts that odor stimulation will give rise to LFPOs within a narrow frequency range that is both common across recording sites (distributed) and independent of odor identity (Laurent and Davidowitz, 1994; Laurent et al., 1996b). LFPOs are theorized to have a relatively stable frequency throughout the life of the response because the mushroom bodies are believed to integrate PN input which is spatially distributed inside the AL, with integration occurring inside a window of time imposed by delayed inhibitory control originating in the LH (Laurent, 2002; Perez-Orive et al., 2002). Each of these predictions was tested by our characterization of LFPOs in the AL.

Odor-driven activity is multi-phased and frequency modulating

Figure 6A illustrates electrode placement into the AL. Four tetrodes are shown, each with 4 electrodes resulting in 16 recorded channels which collected spiking data. The four tetrodes sit in a square formation, with both the left-to-right and top-to-bottom spacing being 150 microns. Within a tetrode, electrodes sit in a diamond formation, with a diagonal ‘nearest neighbor’ distance of 25 microns. Thus the tetrodes are spaced over approximately $\frac{1}{2}$ of the area of the posterior edge of the AL, and are physically arranged as shown in the Figure 6A.

LFP data was also collected in parallel from the highest two and lowest two sites in the array (shown as a small red dot), resulting in four parallel recorded LFP ‘channels’. In Figure 6A, four sample LFP voltage traces in response to a 100 ms odor stimulus (inset red rectangle) are superimposed adjacent to the tetrode which recorded it. Black arrows highlight spontaneous oscillations. Spontaneous LFPOs were observed in all the recordings in this study. This may suggest that LFPOs reflect ongoing circuit-processes of an active system. However, odor driven responses result in larger amplitude LFPOs than those observed during spontaneous activity (See Fig 6A).

This is also evident in Figure 6B, which shows raw LFP traces for 20 presentations of each of 9 different odors. Each odor is identified on the left of the panel. In this case, the voltage is represented as 1 dimensional (i.e. a line) a heat scale with voltage peaks colored red, and valleys colored blue; this is illustrated in the upper left hand corner of the figure. All traces are aligned in time relative to the start of odor presentation (electronically recorded as the time when the odor-laden air valve opened) and stacked as in a spike raster. The duration of the stimulus is highlighted by the inset bracket. This grouping or ‘stacking’ of the 20 repetitions, highlights the consistent aspects of LFPO response to odor. Note first that, even after this procedure, spontaneous oscillations remain visible in all traces as a peppering of red and blue points indicating peaks and valleys in the individual traces that do not align with subsequent traces.

As in Figure 6A, Figure 6B establishes that odor stimulation increases LFPO amplitude. This increase exhibits stimulus specific trial-to-trial coherency for a subset of odors (for example, see A5). Trial-to-trial consistent activity is visible for most odors during the early epoch of response

(e1, ~50-100ms), and for some odors during parts of the later epoch of response starting around 150 ms after response onset (e2). Neurophysiologically, e1 precedes the phase of inhibition commonly called I1 (Christensen et al., 1998). I1 is an odor-driven GABAergic inward Cl⁻ conductance observed in *Manduca* AL PNs which occurs immediately before PN spiking. This suggests that early phase LFPOs during e1 are related to synaptic processing prior to PN spiking. Late epoch (e2) responses occur at least 120 ms after stimulus onset, and show greater odor-to-odor and trial-to-trial variance than e1 responses.

While the voltage traces in Figure 6B give an intriguing glimpse of the aspects of LFPO structure which are consistent trial-to-trial, they do little to highlight the changing nature of LFPO frequency. This is a particularly important problem in cases where oscillations do not align across repeats of a given odor (e.g. A6). Since the TOM predicts that LFPOs should be of a reasonably stable frequency in order to act as a temporal encoding mechanism (Laurent et al 2002), we investigated odor-driven LFPOs via averaged TFRs, such as those shown in Figures 6C and 6D. Figure 6C shows the mean time-frequency response (TFR) for 1-pentanol. This analysis is based on the averaging of 20 individual TFR analyses, each based on a single peristimulus response to 1-pentanol. The oscillatory power of each frequency, at each instant in time has been normalized to unity, and color coded indicating normalized relative power (lowest power – 0—dark blue, highest power – 1—deep red).

This TFR representation makes possible several observations which were not obvious from voltage traces alone (Figure 1B). First, early-phase (e1) activity begins at high frequency (in this case ~100 Hz). Next, the e2 epoch typically starts at ~80 Hz and modulates downward toward ~35 Hz over a span of approximately 350 ms. We observed this frequency modulation (FM) pattern in response to all odors (N=9) across all moths (N=10). This odor-elicited pattern has significantly higher normalized statistical power than spontaneous activity. This indicates that odor-driven LFPOs have higher amplitudes than spontaneous LFPOs. More importantly, however, our approach clearly determines aspects of the oscillatory response which occur at consistent times relative to stimulation, on a trial-to-trial basis. By comparing averaged TFRs to peri-stimulus time histograms of spiking activity (as with TFRs, averaged over 20 repetitions), we see that the LFPO sweep precedes the spiking response (in this case by ~60 ms). As mentioned, this suggests that the early phase of the oscillations represent pre-spiking synaptic activity.

Second, these FM oscillations were typically observed on only one or two of the four LFP recording sites. Figure 6D for example displays TFRs for all 4 simultaneously recorded electrodes in response to 2-octanone. Note that the FM sweep is strongest in electrode 1, present with somewhat reduced power in electrode 2, and absent in electrodes 3 and 4. This implies that the origin of this LFPO pattern is located nearer to tetrode 1 and 2 than 3 and 4. In addition, in Figure 6D, electrode 3, we observe ongoing and spontaneous 20-50 Hz LFPOs. These oscillations are clearly disrupted, or eliminated upon odor stimulation. Taken together, these observations suggest two things. First they indicate that the high-frequency and odor driven oscillations are spatially localized. Second, ongoing oscillations are typically evident in one or two electrodes in ~60% of recorded animals, suggesting multiple sources of oscillations

– namely that ongoing and odor driven oscillations may arise from distinct and spatially segregated sources.

Odor driven FM oscillations are odor-dependent

The TOM predicts that odor-driven oscillations should be odor-tolerant in that all odors should elicit a common frequency in order to provide a stable temporal signal for downstream integration (Laurent and Davidowitz, 1994; Laurent et al., 1996b). However, our TFR analyses clearly indicate that odor-evoked LFPOs vary systematically with the molecular features of the monomolecular odorant features as well as the duration of stimulation used. For example, the LFPOs evoked by four related odors (recordings drawn from the same tetrode in a single animal) are shown in Figure 7A. The peak frequency varies for each odor: 105 Hz for 2-hexanone, 97 Hz for 1-hexanol, 90 Hz for 2-decanone, and 81 Hz for 1-decanol. While the duration of the stimulus was the same for all odors, the duration of the FM sweep was extended considerably longer for the longer chain odors 2-decanone and 1-decanol. In collaboration with Roberto Galan at Case Western Reserve, we implemented a discriminant function analysis on this data and found that the frequency response profile for each of the 9 odors used were statistically unique (Daly et al., 2011). Thus the FM sweep has greater duration for longer carbon chain odors. This could be explained directly as carbon chain length dependence, or indirectly as an effect of odorant volatility, which decreases with increasing carbon chain length.

As mentioned, the duration of exposure to a stimulus also affects the shape of the FM sweep. In two animals, we examined four separate stimulus durations including; 50, 100, 500, and 1000 ms, Figure 7B displays the effect of stimulus duration on LFPOs. The results displayed are from a single recording electrode in response to 2-hexanone. This figure shows that as the duration of the stimulation increase, the duration of the oscillation increases. More importantly, this more prolonged aspect of the oscillatory response continues to modulate downward, although at a far gentler rate. It is possible therefore that prolonged stimulations (i.e. greater than 1000 ms) could result in a stable frequency. However, these prolonged stimuli are inconsistent with naturally occurring odor plumes (Murlis and Jones, 1981).

Spike phase locking is an ongoing and not a stimulus driven effect

The TOM predicts that spiking activity among distributed PNs of the AL will be synchronized by the LFPO (Laurent, 2002). We therefore performed vector strength analysis on individual neural units to examine the phasic relationship between spiking activity and oscillations. We performed this analysis as a function of three peristimulus time epochs (spontaneous -300 to 0 ms; early response 50-350 ms; and late response 350-650 ms post-stimulus). Furthermore, we performed this analysis on two LFPO frequency ranges (25-55 Hz and 55-85 Hz). Here, the expectation is that vector strength should be significantly greater during odor driven responses than to spontaneous activity. As shown in Figure 8 this was not the case. To our surprise and contrary to the TOM, an ANOVA of unitary vector strength as a function of response epoch indicated that spontaneous spiking activity produced significantly greater vector strength values (i.e. more phase locking) than either the early or late post stimulus response epochs ($p < 0.001$). More

specifically, phase locking was greatest during spontaneous activity, dropped significantly during the early response epoch, and then began to recover during the late response epoch (ANOVA; $p < 0.001$). Note that this pattern of results was consistent across the two LFPO frequency bands we considered. These results indicate that phase locking of spikes is an ongoing, not odor driven activity per se and is driven by a broad band of frequencies ranging from 25-85 Hz.

These conclusions are drawn from statistics generated (aggregated) on the level of individual spiking units. We can supplement this analysis by considering vector-strengths for the entire population of spiking units. If the TOM holds – meaning that LFP oscillations are acting as a synchronizing mechanism in the AL, and if vector strength values accurately reflect spiking synchronization, then we should observe that vector strength values should be equal in magnitude whether calculated at the level of individual spiking-units (as discussed above) or when calculated at the level of the entire AL-level population (all spikes and spiking units aggregated). If the entire population level measure shows decay relative to the spiking unit-level means shown in Figure 8A, this implies that units prefer to spike at different phase angles and hence are to some degree desynchronized. Figure 8B shows the population level mean vector strength for the 25-55 Hz frequency band; this analysis was performed separately on 5 individual recordings (shown as separate panels). Figure 8C shows the higher frequency band (55-85 Hz) from the same recordings. In both Figure 8B and 8C, evidence of phase locking, particularly during odor-response (relative to spontaneous) was approximately half of the unitary analysis. This implies that the bulk of units have an idiosyncratic phase angle which when treated as a population adds variance to the population level vector strength measure thereby reducing the overall strength. This implies that as a synchronizing mechanism, the LFPO does a poor job.

BMI application reduces odor driven LFPO strength

The TOM predicts that the oscillations are mediated by the reciprocal dendrodendritic synaptic interactions between LNs and PNs. Figure 9 displays four TFR analyses before BMI (top panels) and TFRs from the same recording sites during BMI application (bottom panel). Note here that BMI reduced odor-driven LFPOs. The degree of loss of power varied significantly by recording from near total cessation of LFPO power (Figure 9 columns 1 and 2) to a more moderate reduction (Fig. 4 panel columns 3 and 4). While a clear reduction in LFPO strength occurs with BMI application, odor-dependent elements of the timing and frequency structure remain visible. For example, note that in columns 3 and 4 of Figure 9, the downward modulating frequency sweep is visible. This contrasts with columns 1 and 2, for which little the frequency content remained.

GABAA receptor blockade disrupts phase locking

We also quantified the effects of GABA_A blockade on vector strengths. Figure 10A displays the same unitary analysis for the same units and recordings as Figure 5 but now after application of BMI. Note that the same relative pattern of significant decrease in vector strength occurred

during the odor driven response. By comparison with the pre BMI data we found that GABA blockade significantly decreased unitary vector strength ($p < 0.001$) although the effect was small and may reflect the invasiveness of the BMI application method, which causes modest physiological disruption. Figure 10B (low frequency band) and Figure 10C (high frequency band) were prepared identically to Figure 9 (thus the upper and lower rows are paired by recording), but reflect data recorded after application of BMI. The decrease in LFPO power results in decreased vector strength values and supports the notion that GABA_A mediated oscillations do influence spike timing, keeping in mind that this is primarily an ongoing effect. That is, as in Figure 9, phase locking during spontaneous activity was higher than during stimulus response.

Vector strength values are greater than chance

A relevant question is whether the observed levels of phase-locking (as measured by vector strength) differ from what might be expected from chance alone. The average vector strength values are relatively low suggesting that oscillations weakly influence spike timing. To address this concern, we employed spike jittering – randomly shifting the position of spikes within a limited time range, in order to remove the phase relationship which might have been present. This analysis was performed on the same three peri stimulus epochs used above. Shown in Figure 11, spike trains were randomly jittered within a specified time range (uniform probability distribution within that range), and the vector strength was recalculated. This yields a measure of the relationship between the amount of jittering and vector strength. Here we show that even a jitter of just ± 1 ms is sufficient to significantly reduce vector-strengths, to approximately half original values (t-test comparison of mean vector strength by epoch; $p < 0.05$). A jitter of ± 5 ms abolished observed vector strengths in any of the three peri-stimulus time epochs. Further jittering does not significantly reduce vector strength values. The slight ‘hump’ in vector strength observed around ± 7 to 9 ms jitter values is an artifact arising from a lingering harmonic of the primary LFPO frequencies used in this analysis.

The vector-strength taken at ± 5 ms or greater jitter thus illustrates the base-line, or vector strength expected purely by chance. The jitter-free vector-strength values are significantly higher in all three epochs. Thus, cells have a modest, though significant, preferred spiking phase relative to the LFPO. Because of the massive number of spikes in our data set ($\sim 10,000$ -to $\sim 15,000$ in each peri-stimulus time epoch), even a 1 ms jitter results in a statistically significant drop in vector strength. Again, a stronger relationship is observed between spikes and oscillations during spontaneous activity than during odor-evoked activity.

Discussion

This research addresses the unsettled, and at times hotly contested, role of time coding in olfactory neural processing. In specific, this research addresses the transient oscillatory model (TOM), a prominent model of olfactory coding. This model proposes an important role for the time domain in olfactory coding. Specifically it has been proposed that PN-PN synchrony occurs transiently, as cells enter and leave the response ensemble. As PNs enter the response ensemble they spike synchronously, with synchrony established by the oscillating extracellular field (LFPO). The TOM makes several predictions for AL processing which we have tested in this study. Among these predictions are that LFPOs should be distributed across the AL in order to synchronize equally distributed PNs. LFPOs should also be stable and consistent across odors, so as to act as faithful timing references which are tolerant to changes in odor and stimulus contexts. Further, odor-stimulation should drive periods of heightened phase-locking in coding PNs. Finally, GABA-A blockade should selectively abolish both LFPOs and PN phase-locking. This research has led to several significant findings which help us elucidate and test the predictions of the TOM.

LFPO stability and consistency

First, in sharp contrast to the TOM, we demonstrate that LFPO frequency is not stable during olfactory response – instead it is strongly frequency modulating. Further, this pattern varies significantly based on the nature the stimulation. The duration of this modulating sweep in frequency was a function of stimulus duration, and stabilized in the later phases of response.

Time-frequency analysis revealed that stimulus-evoked LFPOs consistently initialized at the peak frequency ~60 ms after stimulus onset. Although the nature of the modulation was stimulus-specific, the most commonly observed pattern was for LFPO frequency to initially peak at ~70-100 Hz. The frequency generally modulated down and began to stabilize at ~35-40 Hz at around 350-750 ms after stimulus onset. The duration and frequency ranges of this modulation also varied significantly with stimulus identity.

Two traits of the LFPO response – the timing of high-frequency components, and their subsequent downward sweep in frequency – show intriguing hints of a relationship to the carbon chain length of the stimulating odorant. Although we have not yet established a systematic relationship between chemical parameters of the stimulus and the resultant LFPO structures, a promising hypothesis is that the shape of these structures is partially a function of molecular weight and therefore odor volatility. In addition to odor-dependent variations in frequency, we found LFPOs to vary with the overall state of the system. State-dependence of distributed LFPOs is revealed by a categorical change in frequency spectra upon stimulation. Note that this is distinct from the frequency-modulating effect during stimulation (described above). Simply, the ongoing, spontaneous, non-odor driven activity occurs primarily between 20-55 Hz, while odor-elicited LFPOs occur primarily between 55-85 Hz. Similar frequency shifts have been observed in a range of other species, including monkeys (Rickert et al., 2005) and rats (Tort et al., 2008).

Both PNs and LNs are known to be spontaneously active. In the OB (the vertebrate homolog of the AL), at least a portion of 'fast' network oscillations are known to arise from intrinsic circuitry (Lagier et al., 2004; Neville and Haberly, 2003), arising from spontaneous activity as well as odor-generated stimulation. In the AL of insect, similar 'fast' oscillations are observed. These fast, stimulus driven, and frequency modulating oscillations are believed to correspond to the PN-LN circuit (Buonviso et al., 2003). In the TOM it was expected that these oscillations are responsible for PN-PN synchronization (Stopfer et al., 1997). So it is perhaps likely that the observed stimulus driven 20-55 Hz activity relates to the 'passive' rhythm of spontaneous PN activity, through the same circuitry of reciprocal PN-LN connections within the AL, which plays a prominent role in spontaneous olfactory activity. This disagrees with theories (although not the TOM), which hold that LFPOs reflect ongoing circuit-processes, rather than those which are specifically odor-driven (Kelly et al., 2010). Further, we found that both spontaneous and odor-driven frequency patterns tended to be spatially localized to one or two recording electrodes, but themselves were not co localized. This later point indicates that there is not a single oscillatory mechanism but rather multiple.

Frequency varying LFPOs present a difficulty for the TOM, which propose that part of the downstream meaning of odor-elicited PN spiking activity is partially determined relative to the timing of broadly distributed patterns of activity in the AL or MB. Hence, if we assume that the predictions of the TOM hold, we would expect to see LFPOs whose frequency is reasonably stable as well as independent of odorant identity. Similar to a computer clock cycle, stable frequency oscillations would establish a common timing pattern for all downstream receivers of the distributed output of the AL. In fact the TOM even proposes an integrate-and-reset window arising from both excitatory output from AL to MB and indirect inhibitory input from AL through LH to MB (Perez-Orive et al., 2002). Given the fixed conduction times of neurons, the static integration window this imposes on Kenyon cells in the MB will have very limited room for variance in integration time before the LH input resets the window, and hence there will be limited frequency tolerance. Thus, it is difficult to see how the TOM could cope with rapidly frequency modulating LFPO activity in the AL. Whereas TOM predicts a stable frequency (since it is to act as a timing reference), there are findings which implicate other possible functional roles. For example, it has been suggested that LFP frequency may be a function of the intensity of ORN population response (Ito et al., 2009), rather than an intrinsic coding mechanism.

Odor-driven phase locking

The TOM predicts that distributed oscillatory activity influences PN spiking behavior across the AL, increasing PN-PN synchrony. By examining both TFRs of LFPOs and vector strength analyses of PN activity with LFPOs, we found both confirmation and disagreement with the TOM's predictions. In agreement with the expectations of the TOM, spike timing does have a statistically preferred LFPO phase on the level of individual cells. Preferred phase varied between cells, and with network state. We observed that individual cells exhibit a preferred spiking phase during spontaneous (non-odor evoked) activity. Not all preferred phases were equally likely – there was a tendency for individual cells to prefer to spike in the period just following the peak of the local field potential oscillation. Further, we observed greater

population-level phase-locking during spontaneous PN activity than is attributable to chance alone.

In contrast to TOM predictions, population-level phase locking was significantly reduced by odor-stimulation, because the spike-to-LFPO phase angle of individual units became more variable and perhaps more importantly because the firing rate of cells far exceeds the frequency of the oscillation. PN phase locking also shows an incremental post-stimulus return to pre-stimulus levels of phase-locking. This reduction is found to be true regardless of the LFPO frequency range used, or the time period of response considered (early or late response).

Hence, it does not appear that LFPOs are acting as a synchronizing mechanism during olfactory response. Since it has been shown that odor representation is optimally distinctive in the AL by 240 ms after stimulus onset (Daly et al., 2004b; Staudacher et al., 2009; Wesson et al., 2008) we conclude that LFPO-based synchronization is not playing a role in odor identity coding.

On odor stimulation however, individual spiking units increase in phase-variability because they are spiking at a rate well above the oscillatory frequency (ca.200-250 Hz; see (Christensen et al., 1998) for PN spike frequencies). At a population level, these changes in individual-level spiking resulted in a more uniform distribution of preferred phases, and hence a weaker population vector (or population variance). Physiologically, this could arise because the response of individual PNs to odor consists of a rapid IPSP, followed by sustained and rapid-fire bursting, up to 250 Hz for 50-100 ms in duration (Staudacher et al., 2009). During bursting, action potentials are produced at a rate considerably greater than LFPO frequency. Consequently, because spikes are occurring at all phases of the oscillation cycle it would be difficult or impossible for PN activity to be entrained to any particular phase of LFPO.

GABA-A Blockade

Under the TOM model of AL function, PN timing is shaped by reciprocal connections with laterally inhibiting LNs. LN inhibition is GABAergic, and selectively blocked by experimental application of BMI – which enabled us to test the effects of the LN-PN circuit on PN phase-locking. Not surprisingly loss of inhibition resulted in loss of oscillations. Indeed, we found that BMI application drastically reduced both stimulus-driven and spontaneous LFPOs. Secondly, GABA-A blockade reduced odor-driven phase-locking. However, as observed previously in the BMI-free conditions, phase-locking remained higher in spontaneous activity than in odor-driven activity. Again, we interpret the consistent finding that phase locking is a hallmark of ongoing not odor driven activity. Furthermore, we can place these findings within the context of the functional consequences of GABA receptor blockade and behavioral measures of odor detection and discrimination. GABA blockade did reduce evidence of fine odor discrimination, as measured by stimulus generalization (Mwilaria et al., 2008). However, these studies also show that actual measures of discrimination were broadly disrupted and this was likely attributable to a loss of ability to detect the odor, suggesting that the role of the GABAergic network is to establish a low noise odor signal (i.e. contrast enhanced) to the MB in a manner similar to lateral inhibition in the retina.

References

- Assisi, C., Stopfer, M., Laurent, G., and Bazhenov, M. (2007). Adaptive regulation of sparseness by feedforward inhibition. *Nature Neuroscience* *10*, 1176–1184.
- Bazhenov, M., Stopfer, M., Rabinovich, M., Huerta, R., Abarbanel, H.D., Sejnowski, T.J., and Laurent, G. (2001). Model of transient oscillatory synchronization in the locust antennal lobe. *Neuron* *30*, 553.
- Bhandawat, V., Olsen, S.R., Gouwens, N.W., Schlieff, M.L., and Wilson, R.I. (2007). Sensory processing in the *Drosophila* antennal lobe increases reliability and separability of ensemble odor representations. *Nature Neuroscience* *10*, 1474–1482.
- Buonviso, N., Amat, C., Litaudon, P., Roux, S., Royet, J.-P., Farget, V., and Sicard, G. (2003). Rhythm sequence through the olfactory bulb layers during the time window of a respiratory cycle. *European Journal of Neuroscience* *17*, 1811–1819.
- Christensen, T.A., Waldrop, B.R., Harrow, I.D., and Hildebrand, J.G. (1993). Local interneurons and information processing in the olfactory glomeruli of the moth *Manduca sexta*. *Journal of Comparative Physiology A* *173*, 385–399.
- Christensen, T.A., Waldrop, B.R., and Hildebrand, J.G. (1998). Multitasking in the olfactory system: context-dependent responses to odors reveal dual GABA-regulated coding mechanisms in single olfactory projection neurons. *The Journal of Neuroscience* *18*, 5999–6008.
- Daly, K.C., Christensen, T.A., Lei, H., Smith, B.H., and Hildebrand, J.G. (2004a). Learning modulates the ensemble representations for odors in primary olfactory networks. *Proceedings of the National Academy of Sciences of the United States of America* *101*, 10476–10481.
- Daly, K.C., Wright, G.A., and Smith, B.H. (2004b). Molecular features of odorants systematically influence slow temporal responses across clusters of coordinated antennal lobe units in the moth *Manduca sexta*. *Journal of Neurophysiology* *92*, 236–254.
- Daly, K.C., Galán, R.F., Peters, O.J., and Staudacher, E.M. (2011). Detailed characterization of local field potential oscillations and their relationship to spike timing in the antennal lobe of the moth *Manduca sexta*. *Frontiers in Neuroengineering* *4*.
- Denker, M., Roux, S., Lindén, H., Diesmann, M., Riehle, A., and Grün, S. (2011). The local field potential reflects surplus spike synchrony. *Cerebral Cortex* *21*, 2681–2695.
- Desmaisons, D., Vincent, J.-D., and Lledo, P.-M. (1999). Control of action potential timing by intrinsic subthreshold oscillations in olfactory bulb output neurons. *The Journal of Neuroscience* *19*, 10727–10737.
- Friedrich, R.W., Habermann, C.J., and Laurent, G. (2004). Multiplexing using synchrony in the zebrafish olfactory bulb. *Nature Neuroscience* *7*, 862–871.

- Gray, C.M., and Di Prisco, G.V. (1997). Stimulus-dependent neuronal oscillations and local synchronization in striate cortex of the alert cat. *The Journal of Neuroscience* 17, 3239–3253.
- Ito, I., Bazhenov, M., Ong, R.C., Raman, B., and Stopfer, M. (2009). Frequency transitions in odor-evoked neural oscillations. *Neuron* 64, 692–706.
- Kashiwadani, H., Sasaki, Y.F., Uchida, N., and Mori, K. (1999). Synchronized oscillatory discharges of mitral/tufted cells with different molecular receptive ranges in the rabbit olfactory bulb. *Journal of Neurophysiology* 82, 1786–1792.
- Kelly, R.C., Smith, M.A., Kass, R.E., and Lee, T.S. (2010). Local field potentials indicate network state and account for neuronal response variability. *Journal of Computational Neuroscience* 29, 567–579.
- Krishnan, P., Duistermars, B.J., and Frye, M.A. (2011). Odor identity influences tracking of temporally patterned plumes in *Drosophila*. *BMC Neuroscience* 12, 62.
- Lagier, S., Carleton, A., and Lledo, P.-M. (2004). Interplay between local GABAergic interneurons and relay neurons generates γ oscillations in the rat olfactory bulb. *The Journal of Neuroscience* 24, 4382–4392.
- Laurent, G. (2002). Olfactory network dynamics and the coding of multidimensional signals. *Nature Reviews Neuroscience* 3, 884–895.
- Laurent, G., and Davidowitz, H. (1994). Encoding of Olfactory Information with Oscillating Neural Assemblies. *Science* 265, 1872–1875.
- Laurent, G., Wehr, M., MacLeod, K., Stopfer, M., Leitch, B., and Davidowitz, H. (1996a). Dynamic encoding of odors with oscillating neuronal assemblies in the locust brain. *The Biological Bulletin* 191, 70–75.
- Laurent, G., Wehr, M., and Davidowitz, H. (1996b). Temporal Representations of Odors in an Olfactory Network. *J. Neurosci.* 16, 3837–3847.
- Laurent, G., Stopfer, M., Friedrich, R.W., Rabinovich, M.I., Volkovskii, A., and Abarbanel, H.D. (2001). Odor encoding as an active, dynamical process: experiments, computation, and theory. *Annual Review of Neuroscience* 24, 263–297.
- MacLeod, K., Bäcker, A., and Laurent, G. (1998). Who reads temporal information contained across synchronized and oscillatory spike trains? *Nature* 395, 693–698.
- Mombaerts, P., Wang, F., Dulac, C., Vassar, R., Chao, S.K., Nemes, A., Mendelsohn, M., Edmondson, J., and Axel, R. (1996). The molecular biology of olfactory perception. In *Cold Spring Harbor Symposia on Quantitative Biology*, pp. 135–145.
- Murlis, J., and Jones, C.D. (1981). Fine-scale structure of odour plumes in relation to insect orientation to distant pheromone and other attractant sources. *Physiological Entomology* 6, 71–86.
- Mwilaria, E.K., Ghatak, C., and Daly, K.C. (2008). Disruption of GABAA in the insect antennal lobe generally increases odor detection and discrimination thresholds. *Chemical Senses* 33, 267–281.

- Neville, K.R., and Haberly, L.B. (2003). Beta and gamma oscillations in the olfactory system of the urethane-anesthetized rat. *Journal of Neurophysiology* 90, 3921–3930.
- Perez-Orive, J., Mazor, O., Turner, G.C., Cassenaer, S., Wilson, R.I., and Laurent, G. (2002). Oscillations and sparsening of odor representations in the mushroom body. *Science* 297, 359–365.
- Pinching, A.J., and Powell, T.P.S. (1971). The neuropil of the glomeruli of the olfactory bulb. *Journal of Cell Science* 9, 347–377.
- Rabinovich, M., Volkovskii, A., Lecanda, P., Huerta, R., Abarbanel, H.D.I., and Laurent, G. (2001). Dynamical encoding by networks of competing neuron groups: winnerless competition. *Physical Review Letters* 87, 068102.
- Rasch, M.J., Gretton, A., Murayama, Y., Maass, W., and Logothetis, N.K. (2008). Inferring spike trains from local field potentials. *Journal of Neurophysiology* 99, 1461–1476.
- Rickert, J., de Oliveira, S.C., Vaadia, E., Aertsen, A., Rotter, S., and Mehring, C. (2005). Encoding of movement direction in different frequency ranges of motor cortical local field potentials. *The Journal of Neuroscience* 25, 8815–8824.
- Rodriguez-Gil, D.J., Hu, W., and Greer, C.A. (2013). Dishevelled Proteins Are Associated with Olfactory Sensory Neuron Presynaptic Terminals. *PloS One* 8, e56561.
- Sachse, S., and Galizia, C.G. (2002). Role of inhibition for temporal and spatial odor representation in olfactory output neurons: a calcium imaging study. *Journal of Neurophysiology* 87, 1106–1117.
- Schoppa, N.E. (2006). Synchronization of olfactory bulb mitral cells by precisely timed inhibitory inputs. *Neuron* 49, 271–283.
- Shang, Y., Claridge-Chang, A., Sjulson, L., Pypaert, M., and Miesenböck, G. (2007). Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe. *Cell* 128, 601–612.
- Staudacher, E.M., Huetteroth, W., Schachtner, J., and Daly, K.C. (2009). A 4-dimensional representation of antennal lobe output based on an ensemble of characterized projection neurons. *Journal of Neuroscience Methods* 180, 208–223.
- Stopfer, M., and Laurent, G. (1999). Short-term memory in olfactory network dynamics. *Nature* 402, 664–668.
- Stopfer, M., Bhagavan, S., Smith, B.H., and Laurent, G. (1997). Impaired odour discrimination on desynchronization of odour-encoding neural assemblies. *Nature* 390, 70–74.
- Stopfer, M., Jayaraman, V., and Laurent, G. (2003). Intensity versus identity coding in an olfactory system. *Neuron* 39, 991–1004.
- Tort, A.B., Kramer, M.A., Thorn, C., Gibson, D.J., Kubota, Y., Graybiel, A.M., and Kopell, N.J. (2008). Dynamic cross-frequency couplings of local field potential oscillations in rat striatum and hippocampus during performance of a T-maze task. *Proceedings of the National Academy of Sciences* 105, 20517–20522.

Tripathy, S.J., Peters, O.J., Staudacher, E.M., Kalwar, F.R., Hatfield, M.N., and Daly, K.C. (2010). Odors pulsed at wing beat frequencies are tracked by primary olfactory networks and enhance odor detection. *Frontiers in Cellular Neuroscience* 4.

Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A., and Axel, R. (1999). A Spatial Map of Olfactory Receptor Expression in the *Drosophila* Antenna. *Cell* 96, 725–736.

Wehr, M., and Laurent, G. (1996). Odour encoding by temporal sequences of firing in oscillating neural assemblies. *Nature* 384, 162–166.

Wesson, D.W., Carey, R.M., Verhagen, J.V., and Wachowiak, M. (2008). Rapid encoding and perception of novel odors in the rat. *PLoS Biology* 6, e82.

Wilson, D.A., and Leon, M. (1988). Spatial patterns of olfactory bulb single-unit responses to learned olfactory cues in young rats. *Journal of Neurophysiology* 59, 1770–1782.

Wilson, R.I., Turner, G.C., and Laurent, G. (2004). Transformation of olfactory representations in the *Drosophila* antennal lobe. *Science Signaling* 303, 366.

Appendix

Hilbert Transform

To calculate the phase of LFPOs, we used the Hilbert transform to generate the complex analytic signal of the LFP waveform. Mathematically, the Hilbert transform of the waveform $f(t)$ at time t is defined by:

$$H(t) \equiv \frac{1}{\pi} \int_{-\infty}^{\infty} \frac{f(x)}{t-x} dx$$

The transform is usually calculated via the following equation (given that $*$ represents the convolution operator):

$$H(t) = \frac{1}{\pi t} * f(t)$$

Taking the instantaneous angle (imaginary part) of this value yields the instantaneous phase, $\varphi(t)$, while the absolute value (or real part) is equal to the original signal, $f(t)$.

$$\varphi(t) = \text{Arg}(H(t))$$

Directional Statistics

Calculating statistical moments (~mean and variance) of angular data, such as phase, requires correction to the conventional statistical equations. Let us denote the phase of a particular spike as φ_j (calculated as above) for each of the N spikes in the population, and i as the imaginary unit. Then we may calculate the sample mean ($\bar{\varphi}$) and variance (σ^2) as:

$$\bar{\varphi} = \text{Arg}\left(\frac{1}{N} \sum_{j=1}^N e^{i\varphi_j}\right)$$

$$\sigma^2 = 1 - \left| \frac{1}{N} \sum_{j=1}^N e^{i\varphi_j} \right| = 1 - R$$

The value R is the vector strength (~vector length) in polar plots of phase. $R = 0$ indicates no preferred phase, $R=1$ indicates zero variance.