Relating Neural Dynamics to Olfactory Coding and Behavior

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Relating Neural Dynamics to Olfactory Coding and Behavior
by
Chao Li

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Sensory stimuli often evoke temporal patterns of spiking activity across a population of neurons in the early processing stages. What features of these spatiotemporal responses encode behaviorally relevant information, and how dynamic processing of sensory signals facilitates information processing are fundamental problems in sensory neuroscience that remain to be understood. In this thesis, I have investigated these issues using a relatively simple invertebrate model (locusts; *Schistocerca americana*).

In locusts, odorants are transduced into electrical signals by olfactory sensory neurons in the antenna and are subsequently relayed to the downstream neural circuit in the antennal lobe (analogous to the olfactory bulb in vertebrates). We found that the sensory input evoked by an odorant could vary depending on whether the stimulus was presented solitarily or in an overlapping sequence following another cue. These inconsistent sensory inputs triggered dynamic reorganization of ensemble activity in the downstream antennal lobe. As a result, we found that the neural activities evoked by an odorant pattern-matched across conditions, thereby providing a basis for invariant stimulus recognition. Notably, we found that only the combination of neurons activated by an odorant was conserved across conditions. The temporal structure of
the ensemble neural responses, on the other hand, varied depending on stimulus history: synchronous ensemble firings when stimulated by a novel odorant compared to asynchronous activities induced by a redundant stimulus. Furthermore, these neural responses were refined on a slower timescale (on the order of minutes, i.e. happening over trials) such that the same information about odorant identity and intensity was represented with fewer spikes. We validated these interpretations of our physiological data using results from multiple quantitative behavioral assays. In sum, this thesis work provides fundamental insights regarding behaviorally important features of olfactory signal processing in a relatively simple biological olfactory system.
Chapter 1: Introduction

1.1 The Anatomy of Olfactory System

All organisms perceive the external world through sensory systems that have evolved to extract useful information for their survival and procreation. In particular, olfaction is a primary sensory modality for many organisms as it serves several important purposes such as sampling environment for food, social cues and predators. Compared to other sensory modalities, relatively less is understood about the sense of smell. For example, the dimensions of the olfactory space [1], the molecular features sensed by olfactory receptor neurons, and whether there exists a determinable relationship between the structure of a molecule and the overall percept it might generate are all fundamental problems that remain to be understood.

Odorants are detected by the olfactory receptor neurons (ORNs) that convert chemical cues into electrical signals. In insects, the ORNs are typically located in the antennae. In some cases they can also be found on maxillary palps, a sensory appendage close to the mouth parts [2]. The current dogma is that in insects, each ORN selectively expresses a specific olfactory receptor (OR) gene [3], along with a broadly expressed co-receptor [4, 5]. The activation of a receptor neuron is completely determined by the olfactory receptor gene expressed [6, 7]. Furthermore, using heterologous expressions, it has been shown that the olfactory receptors primarily function as ligand-gated ion channels in insects [8]. Also, it is worth noting that a single odorant typically activates a unique combination of receptor neurons, and individual neurons can be activated by different odorants.

The sensory neuron responses are then transmitted to the second relay station: the antennal lobe (AL). Here, the axons of ORNs expressing the same OR gene converge onto one or
a few spherical structures of neurophil, called glomeruli (Fig. 1.1)[9-11]. This convergence of redundant ORN inputs has been shown to be important for increasing the signal to noise ratio [12]. In each glomerulus, ORN axons make excitatory synapses onto projection neurons (PNs), the principal neurons of the AL. Within glomeruli, PNs form reciprocal dendrodendritic interactions with GABAergic, axon-less local neurons (LN). Both widely-branching and spatially-restricted LN have been found in the AL[13], suggesting a wide range of interactions between these cell types. In addition, recent studies have reported the presence of cholinergic local neurons that are also able to provide lateral excitation through electrical synapses[14, 15].

PNs, the sole output neurons of the AL, send signals to the higher brain centers: the mushroom body (MB) and the lateral horn (LH). PNs send the axonal tracts to the MB calyx, a hemispherical protuberance, where PN axons broadly branch throughout the area. This widespread projection from the AL to the calyx of the MB form excitatory synaptic interaction with intrinsic neurons, termed Kenyon cells (KCs) [16, 17]. Typically, the connectivity ratio of a single PN to all KCs is 50%, i.e. a single PN can send synaptic input to half of the KC population. KCs also receive inhibitions in a feed-forward manner from a giant GABAergic interneuron[18].
Figure 1.1 The anatomy of insect olfactory system. In the insect olfactory system, the cholinergic olfactory receptor neurons (ORNs) expressing the same receptors (indicated by the same color) send their axons to the downstream structure in the antennal lobe (AL). Within glomeruli, excitatory projection neurons (PNs) and inhibitory local neurons (LNs) form extensive dendrodendritic interaction. Further, PNs provide the sole output of the AL to higher olfactory center (reproduced as is from [19]).

The invertebrate olfactory system shares many structure and functional similarities with the vertebrate olfactory system [19]. In mammals, ORNs are located in the olfactory epithelium within the nasal cavity at the roof of the nose [20]. Each ORN expresses one type of ORs that belong to a large family of seven-transmembrane G-protein coupled receptors (GPCRs) and are activated through a second messenger mechanism. These glutamatergic ORNs project their axons onto glomeruli in the olfactory bulb (OB; analogous to the AL; Fig. 1.2). Here again the ORNs expressing the same gene converge onto a single or few glomeruli. In the glomerular
layer, the glutamatergic mitral and tufted (M/T) cells (analogous to PNs in the insect antennal lobe) form synaptic connections with the inhibitory periglomerular cells that release GABA, dopamine or both[21]. In the same layer, GABAergic short axon cells have also been identified to form inter-glonerular connections with M/T cells [22]. These interneurons contribute to inter-glonerular interactions are also generally termed as juxtaglomerular cells. Below the glomerular layer lies the mitral and granule cell layer, where M/T cells form dendrodendritic interactions with GABAergic granule cells that are axon-less cells like LNs in the invertebrate AL.

M/T cells in the OB send their output through the olfactory tract to several different cortical targets in the vertebrate brain, such as anterior olfactory nucleus, piriform cortex, cortical amygdala, etc.[23]. These areas integrate olfactory information with other sensory modality through reciprocal connections with different cortical areas [24].
Figure 1.2 The anatomy of vertebrate olfactory system. For vertebrates, glutamatergic ORNs expressing the same receptors (indicated by the same color) send their excitatory axons to their post-synaptic target in the olfactory bulb (OB), where different types of cells, including glutamatergic mitral and tufted (M/T) cells, GABAergic short axon cells, periglomerular cells, and granule cells, form synaptic interaction. M/T cells provide the output of the OB to higher olfactory center. GL: glomerular layer. MCL: mitral cell layer. GRL: granule cell layer. Dashed line on the right indicates the boundary of OB (reproduced as is from [19]).

1.2 Olfactory Coding Principles
Time and space are fundamental neural coding dimensions. Sensory cues, even stationary ones, often activate an ensemble of neurons with a precise temporal structure. Determining what
features of a stimulus are encoded by the active set of neurons (‘spatial code’) and what aspects
are represented in their temporal structure (‘temporal code’) is a fundamental problem in systems
neuroscience. Alternatively, these two dimensions may not independently encode information. In
this case, the joint spatiotemporal patterns of spiking activity could provide a large coding space
for representing stimuli\[25, 26\]. Identifying the right coding scheme employed by a sensory
system is essential for determining the rules that govern how stimulus-evoked neural activity is
translated to a behavioral response. In this section, I discuss some of the popular olfactory coding
principles that have been proposed.

1.2.1 The Spatial Code

Stimulus information can be represented in a spatially distributed manner i.e. based on
the combination of neurons activated \[27, 28\]. As mentioned earlier, an odorant activates a
selective subset of receptor types. Thus distinct odorants activate distinct sensory neurons, and
thereby provide spatially segregated input to the AL or OB\[29-32\]. This scheme is referred to as
a spatial code, and can be used to encode information that correlates with certain molecular
features of an odorant \[33, 34\].

Such spatial coding strategy has also been reported to encode information about odor
valence (attractant or repellent odors) \[35, 36\]. In flies, attractant odors activate the medial
regions of the AL, whereas repellents activate the lateral regions\[36\]. Also in flies, attractant
cues that are delivered unilaterally activate ipsilateral AL, whereas unilateral delivery of
repellents excites both antennal lobes \[35\].

In some cases, an ORN can only be activated by one or a narrow range of odorants, and
therefore only a specific region of the AL or OB is activated for representing particular cues.
This strategy is an extreme case of spatial coding, and referred to as a ‘label-line’ scheme. For
example, investigators found that in insects like flies and moths, specialized ORNs in male insect antenna can detect components of sexual pheromones released by female insects[37, 38], and innervate a sexually dimorphic glomerulus in the AL[39, 40].

1.2.2 The Temporal Code

Odor-evoked neural activities are temporally patterned. These neural responses are considered a ‘temporal code’ if they change on a timescale that is different than the timescales of stimulus variations and if they convey useful information about the stimulus [41]. To describe the role of temporal coding in olfaction, several hypotheses have been proposed.

Early in 1995, Hopfield proposed the latency coding principle[42]. Neurons that receive stronger input have shorter response latency, and consequently the system converts input strength into spike timing. In theory, this coding strategy of using spike latency allows for rapid odorant identification and behavioral reaction. Moreover, this hypothesis is supported by experimental results. In rodents, the latency of glomerular odor responses is stimulus-specific [43, 44], and as odor intensity increases, the spike timing of M/T cells with respect to respiration cycle advances [45-47].

The Hopfield’s hypotheses suggest one way to extract information in temporal patterns using spike latency. However, a primary concern about such a coding scheme is the intrinsic variability of latency to first-spike, which may compromise the decoding efficiency [48]. In addition, temporal dynamics in the olfactory system are more complex and typically go beyond the first spike latency[28]. Further, encoding and decoding stimulus-specific information using latencies of an ensemble of neurons would be a big challenge. Therefore, alternative strategies are required for extracting information from such a neural representation.
Odorants entrain oscillatory synchronization of principal neuron activities in the AL and OB[26, 49-54]. This neural synchronization gives rise to local field potential (LFP) signals, which exhibit unique oscillatory characteristics (e.g. amplitude, frequency) to represent odors identity and intensity. For example, as odor concentration increases, the oscillatory amplitude increases while the oscillatory frequency remains unchanged [49]. Investigators also found that beta oscillations (15-30 Hz) in the rat OB can only be evoked by some organic solvents (e.g. xylene) but not strong odors (e.g. ammonia) [55]. In addition, within each LFP oscillatory cycle, a subgroup of principal neurons is transiently co-activated [18, 51, 56-58], which are informative about odor identity. In locusts for example, subsets of PNs are reliably co-active during particular oscillatory cycles in response to an odorant, and this co-activation pattern varies with different odorants [26]. Moreover, individual or subsets of neurons in the AL or OB appear to be phase-locked the oscillation cycles, i.e., the timing of action potentials relative to a specific reference during each cycle is fixed [26, 51, 58, 59]. Notably, a single M/T cell in the zebrafish OB can exhibit phase-locked response patterns to some odorants but not for others [60].

Besides oscillatory dynamics, an odor stimulus also generates pronounced slow patterning in the AL or OB [26, 51, 61]. These odor-evoked population neural responses in AL and OB are not static and contain periods of spiking and non-spiking activities [62-64]. Typically, the population neural responses change more dramatically after the odor onset and offset, and are referred to as on- and off-transient periods (Fig. 1.3a,b). For a prolonged odor puff, between these two transient periods, the population neural activity becomes less intense and converges onto a stable pattern of spiking, referred to as steady state. This steady state is typically reached within 1~1.5 s of odor onset [65-68].
Figure 1.3 Response dynamics of the olfactory systems. (a) The odor-evoked principal neuron responses in the antennal lobe are elaborate and change most rapidly (i.e. greater velocity of ensemble firing patterns) following stimulus onset and offset. These response periods have been referred to as ‘on transient’ and ‘off transient’ responses, respectively. For lengthy odor presentations, the antennal lobe activity converges to a steady state within ~ 1.5 s of stimulus onset. Black traces indicate response to individual odors and their response average is shown in red. (b) Similar to panel a, the same response dynamics are observed in the vertebrate olfactory bulb (reproduced as is from [68]).
Different analytical approaches have been used to qualitatively and quantitatively analyze information content of these population neural responses. One approach that is particularly intuitive is through dimensionality reduction. In this analysis, the response of $N$ neurons are treated as an $N$-dimensional response and binned in non-overlapping time bins and projected onto fewer dimensions (typically 2 or 3) for the purposes of visualization. Here time and space dimensions are jointly considered for representing odors, which is referred to as a spatiotemporal code. By visualizing the spatiotemporal activity in two or three dimensions that approximates the information content of high-dimensional responses, how responses vary with odor identity and intensity can be examined. For example, Fig. 1.4 shows the neural activity of 110 PNs responding to three different odorants at four concentrations, in a 3-dimensional space [69]. As seen, three different odors generate response traces that span distinct subspaces, and traces evoked by the same odor with increasing concentrations occupy the same region with increasing span and length.

Moreover, this analytical technique allows visualization of responses during different phases of pronounced odor-evoked response dynamics shown in Fig. 1.3. Note that these odor response trajectories depart rapidly from the origin quickly following odor onset and slow down as they approach steady state regions (Fig. 1.5a). After stimulus termination, the response trajectories again accelerate and evolve into a different region before returning to baseline levels (i.e. off-transient state; see Fig. 1.5b).
Figure 1.4 Trajectories of odor-evoked spatiotemporal antennal lobe activity. (a) Spatiotemporal neural activities are visualized as response trajectories. Dimensionality reduction is performed through local linear embedding (LLE) technique. A total of 110 PN responses to three different odorants delivered at four concentrations are shown. Different stimuli evoke trajectories that occupy distinct regions or subspaces. The neural trajectories evoked by the same odor at different concentrations evolve along the same direction but the length of the trajectories monotonically varies with increase in intensity (b) Odor trajectories representing the same stimulus with increasing concentration levels are shown. Arrows indicate the direction of trajectory evolution over time (reproduced as is from [69])
Analysis of spatiotemporal neural activity in the insect AL and vertebrate OB reveals that the time-varying response patterns are maximally informative during transient phases of neural activity[66], and the discriminability of odors (i.e., the distance between odor trajectories) is greatest during the transient phases rather than during the converged steady state (Fig. 1.6a,b) [66]. Consistent with these physiological observation, behavioral data indicate that animals are capable of discriminating between ‘similar’ odors (a relatively difficult task), within a few hundred milliseconds of stimulus onset [70-73]. Taken together, it can be interpreted that neural activity during on-transient state may be more appropriate to encode odor identity rather than the steady state responses. Further, since brief pulses may require relatively stable stimulus conditions[65, 66, 74], and brief pulses may not evoke a steady-state response, the relevance of the neural activity for olfactory coding and processing is unclear.
Figure 1.5 Odor trajectories of glomerulus activity. (a) Odor trajectories based on glomerular activity observed in a single honeybee are shown for four different odors. Two independent trials are shown for repeatability. Note that the trajectories depart rapidly from their baseline and slow down when they approach the steady state. (b) The same odor trajectories as in panel a (solid lines) with off-transient responses also shown (dashed lines). (reproduced as is from [65])
The prolonged stimulus-evoked activity appears to be more a liability than a feature, as it can potentially interfere with and corrupt the olfactory system’s response to other chemical cues in the environment. Two previous studies\cite{75, 76} that examine this issue report contrasting results. While pulses of the same stimulus generate ensemble activity that is reliable and repeatable at the population level, overlapping pulses of different stimuli create spatiotemporal responses that interfere with each other, thereby making them unpredictable. Whether each dynamic state aids or hinders the rapid odor detection in multi-stimulus environments still remains unclear.

After the odor is terminated, another bout of firings follow and are termed as off-transient dynamics. Interestingly, the offset response patterns in the AL are spatially distributed and can be odor-specific (\textbf{Fig. 1.5c,d}) \cite{66, 77}. However, a systematic study of off-transient states and its behavioral relevance are not fully understood \cite{78, 79}. Therefore, further examination of functions and mechanisms underlying the olfactory offset responses is needed.

\textbf{Figure 1.6 Different phases of odor-evoke response dynamics.} (\textbf{a}) Trajectories (3 s duration) for two different odors are plotted using the same axes. \textit{B} indicates the baseline activity, and the fixed point (FP) are encircled. (\textbf{b}) A schematic of idealized trajectories for two different odors. Arrows indicate direction of trajectory. Two trajectories are maximally separated from each other during the on-transient responses (\textit{m1} and \textit{m2}), and reach their fixed points (steady state; \textit{F1} and \textit{F2}) that are also contained in odor-specific regions (reproduced as is from \cite{66})
So far we have considered the stimulus-evoked neural dynamics within a single trial. In addition to changes that happen during and immediately following odor presentation, odors can also induce dynamic change patterns across repeated experimental trials [80, 81]. At the peripheral stage of sensory processing, sensory neuron responses are very consistent and do not vary much across trials [82, 83]. However, stimulus-evoked responses become more variable when the stimulus is repeatedly presented [84, 85]. Typically, this trial-to-trial response variability is considered as intrinsic synaptic noise of the system [86, 87], or information unrelated to the stimuli [88, 89]. Nevertheless, experimental results suggest that it can also play a critical role in information processing [80, 90]. For example, when a locust is exposed to the same odor stimulus multiple times, the total PN spike counts decrease while the oscillatory power of LFP signals increases [80]. However, what computational functions does this slow dynamics help achieve remains unclear. Furthermore, do these changes interfere with how odor identity and intensity is encoded needs to be determined.

1.3 Behavioral Relevance of Olfactory Coding

What are the rules that govern how neural representations of a given stimulus are translated into appropriate behavioral responses? A number of behavioral assays using different insect models have been developed to study odor-evoked behavior [36, 91-95] and understand their neural basis [96-102]. For example, the navigational mechanism used by moth (Manduca sexta) to localize odor-sources have been studied using wind-tunnel experiments (Fig. 1.7a). In this assay, parameters such as wing fanning, upwind flight duration, time spent during close hover, source contact, and abdomen curl (a typical mating posture), were observed and analyzed to understand odor source localization and pheromone-tracking behavior [100, 103]. Further, the flight path can be recorded and compared before and after pharmacological manipulation of
neural activity in a select olfactory region [100]. This behavioral assay allows us to investigate the relationship between physiological responses and innate behavioral reaction. Another popular assay used to study the innate behavioral preference for an odorant is the T-maze assay (Fig. 1.7b). For example, fruit flies (Drosophila melanogaster) often avoid a T-maze arm containing CO₂, and are attracted to an arm containing apple cider vinegar [98]. Such assays are often used to investigate neural coding strategies for odor valence (attractant and repellent)[36, 98].

To study how olfactory learning impacts behavioral responses, variants of the Pavlovian conditioning paradigm are used. One such paradigm is the proboscis extension (PE) conditioning, which is used to assess the odor discrimination capability of insects like bees and moths [99, 104]. These insects are trained in an appetitive-conditioning paradigm to associate an odor (conditioned stimulus; CS) with a food reward, and then tested with either a conditioned or unconditioned stimulus in an unrewarded test phase. Selective PE is used as a measure of the olfactory memory acquisition (Fig. 1.7c). Similarly, in locusts (Schistocerca gregaria), which is another popular model for studying olfactory coding principles, a variant of the appetitive-conditioning paradigm has been developed to study odor driven behavior [92]. Here, the opening of the locusts’ maxillary palps (palp opening response) is used as an indicator of the acquired olfactory memory (Fig. 1.7d).

We note that comparing the behavioral responses of animals with physiological responses measured simultaneously or in near-identical conditions allow us to identify the rules that govern how stimulus-evoked neural activity gets translated to a behavioral response.
Figure 1.7 Behavioral assays to probe olfactory behavior in different insect models. (a) Odor tracking behavior of moths are typically assessed in wind tunnel experiments. The odor-modulated flight path of individual moths is recorded and analyzed. (b) A schematic of the T-maze assay for fruit flies is shown. Flies movement towards and away from an odor arm is interpreted as attraction or repulsion evoked by the odorant tested. (c) The proboscis extension conditioning tests in honeybees. The insects are trained in a Pavlovian paradigm to specifically associate an odorant (conditioned stimulus; CS) with a food reward (usually sugar water). The selective extension of the proboscis is used as an indicator of the acquired memory. (d) Palp opening responses in locusts. As with honeybees, selective opening of the maxillary palps is used as a measure of acquired olfactory memory (reproduced as is from [101]).

1.4 Thesis Outline

The overall organization of this thesis is as follows: All experimental and analytical methods used in this dissertation are presented in detail in Chapter 2. For parametric analyses, justification for selected values is also provided here. In Chapter 3, I examine how the neural dynamics in the antennal lobe allow for robust stimulus representation in multi-signal environments. Our novel stimulation protocols, behavioral and analytic procedures are discussed here. Finally, I propose and validate a new ‘attractor theory’ for odor coding. Some of the work presented in this chapter has already been published in Nature Neuroscience (2013). In Chapter 4, I decouple and determine what behavioral relevant information is encoded in spatial and
temporal dimensions. This work has also been published in Nature Communications (2015). Finally in Chapter 5, I investigate the role of response changes across trials using a novel data decomposition approach. An integrated conclusion of this work is discussed in Chapter 6.
Chapter 2: Methods

2.1 Experimental Procedures

**Odor Stimulation:** Odor stimuli were delivered using a standard procedure as described in earlier works[75]. Briefly, odor solutions were diluted in mineral oil to achieve different concentration levels by volume. The diluted odor solution was placed in a 60 ml glass bottle with separate inlet and outlet lines. A pneumatic picopump (WPI Inc., PV-820) was used to displace a constant volume (0.1 L min$^{-1}$) of the static headspace above the odorants into a desiccated air stream (0.75 L/min) that was directed at the locust antenna. A vacuum funnel placed right behind the locust preparation continuously removed the delivered odorants. The following odorants were used in this thesis: 2-octanol(2oct), hexanol(hex), cyclo-hexanone(chex), 2-heptanone(2hep), benzaldehyde(bzald), isoamyl acetate(iaa), hexanal(hxa), geraniol(ger), citral(cit), mint and apple. Each stimulus condition was presented multiple times in a pseudo-randomized manner (blocks of 10 or 25 trials) with 60 s inter-trial intervals. Apple (apple green fragrant oil) and mint (peppermint supreme essential oil) were obtained from New Directions Aromatics Inc. All other odorants were obtained from Sigma-Aldrich.

**Olfactory Electrophysiology:** Electrophysiological experiments were performed using locusts (*Schistocerca americana*) raised in a crowded colony. Young adults with fully developed wings (post-fifth instar) of either sex were used. Olfactory receptor neuron (ORN) recordings were made from different sensilla types in an intact but immobilized locust antennae as described previously (Fig. 2.1a) [67]. The antenna was stabilized using wax and a reference electrode (Ag/AgCl wire) was inserted into the locust gut. Single sensillum recordings were made using saline-filled glass micropipettes (~10 μm diameter, 5–10 MΩ) that were inserted into the base of the sensillum. Acquired signals were amplified using a differential amplifier (Grass P55), filtered
between 0.3–10.0 kHz, acquired at 15 kHz sampling rate (PCI-MIO-16E-4 DAQ cards; National Instruments). Multi-unit single sensillum recordings were spike-sorted off-line using Spike-o-Matic software[105] implemented in IGOR Pro (Wavemetrics).

To monitor activity in the antennal lobe and in the mushroom body, locusts were immobilized with both antennae intact, and the brain was exposed, desheathed, and superfused with locust saline at room temperature[106]. In the antennal lobe, multi-unit tetrode recordings were made using 16-channel, 4×4 silicon probes (NeuroNexus). Similar recordings were made in the mushroom body by inserting custom-made twisted wire tetrodes (Nickel Chromium wire, RO-800, Kanthal Precision Technology). KC recordings were made from the superficial layers of the mushroom body that only contains KC somata[18]. Mushroom body recording sites were selected by determining whether a particular location yielded any KC that responded to at least one of the odors tested. All multi-unit electrodes were electroplated with gold to obtain impedances in the 200–300 kΩ range. A custom made 16-channel amplifier (Biology Electronics Shop; Caltech, Pasadena, CA) was used to collect both PN and KC data at 15 kHz. The data was amplified at 10 k gain, filtered between 0.3 to 6 kHz ranges and saved using a LabView data acquisition system (Fig. 2.1b).

A visual demonstration of these multi-unit extracellular recording techniques is now available online[107].
Figure 2.1 Olfactory electrophysiology. (a) A schematic of the single sensillum recording approach. The antenna is immobilized and electrode of saline-filled glass micropipette is inserted into the base of the sensillum. Raw extracellular voltage traces showing responses of ORNs housed in a single sensillum to a 4 s odor pulse is shown (indicated by the gray box). (b) A schematic showing the antennal lobe (AL) and mushroom body (MB) recording configuration. In the antennal lobe, multi-unit tetrode recordings were made using 16-channel, 4×4 silicon probes. KC recordings were made from the superficial layers of the mushroom body that only contains KC somata. Representative raw extracellular traces from a multiunit AL and KC recording are shown. As in the sensilla recording a 4 s odor pulse was applied during the time period indicated by the gray box.

ORN, PN, KC Spike-Sorting: In our single sensillum recordings, spiking events were identified as voltage peaks above a pre-set threshold (usually 2–5 standard deviations). We noticed that the ORN spike amplitude could change somewhat as they adapted to odors. To deal with this issue, while sorting these ORN spikes, we allowed each cell cluster to include events of variable amplitude as long as different sorted units remained well separated (by at least five times noise
standard deviation). Additionally, to be considered single units, less than 20% of spikes associated with the identified unit were allowed to have an interspike interval less than 20 ms. A similar procedure was followed for PN and KC spike-sorting. To allow assignment of recorded spikes to unique cell sources, spike-sorting was done offline using the best three or four channels recorded and conservative statistical principles. Examples of ORN, PN and KC spike-sorting are shown in Fig. 2.2. To identify single units the following criteria were used: cluster separation > 5 noise standard deviations, and number of spikes within 20 ms < 6.5%, and spike waveform variance < 6.5 noise standard deviations. Using this approach a total of 725 PNs recorded from 70 locusts were used in Chapter 3, 844 PNs collected from 88 locusts were used for Chapter 4, and a total of 244 projection neurons were identified from 35 locusts in Chapter 5.
a  ORN spike sorting

![ORN spike sorting](image)

b  PN spike sorting

![PN spike sorting](image)

c  KC spike sorting

![KC spike sorting](image)
Figure 2.2 Examples of ORN, PN and KC spike-sorting. (a) An example of ORN recording and spike-sorting. (Left panel) Raw extracellular trace showing response of a single ORN. (Middle panel) Individual ORN spike events (black) and their mean (red). (Right panel) Inter-spike interval distribution for the identified ORN. (b) An example of PN spike-sorting. (Left panel) Extracellular waveforms from four independent channels of a tetrode are shown for all spiking events corresponding to two simultaneously recorded PNs. Individual events (black), mean (red), and s.d. (blue) are shown for both cells. (Right panel – top) Histograms obtained by projecting high-dimensional PN event representations (180 dimensional vector obtained by concatenating signals from all electrodes) onto the line connecting their means. To be considered a well-isolated unit, as in this case, a bimodal distribution with cluster centers separated by at least five times the noise s.d. is expected for every pair of simultaneously recorded cells. (Right panel – bottom) Distributions of inter-spike intervals are shown for these two PNs. (c) Similar plot showing an example for KC spike-sorting.

2.2 Neural Data Analysis

Peri-stimulus Time Histograms (PSTH): Spike trains of each PN were segmented into 50 ms or 100 ms non-overlapping time bins, summed and smoothed by a 3 or 5 point average zero-phase digital filter. Average across trials and cells were computed to obtain population-level PSTHs.

Excitatory PN Response Categorization: For a projection neuron to be considered as ‘excitatory’ responsive to an odor, the following two independent criteria had to be satisfied: (i) amplitude criterion: odor-evoked neural activity (average over trials) in at least one of the time bins during odor presentation window must exceed 6.5 standard deviations of average baseline activity (average activity during a 2 s pre-stimulus window), and (ii) reliability criterion: the amplitude criterion has to be met in at least 50% of total trials.

Inhibitory PN Response Categorization: The same criteria used to determine an excitatory PN response was followed here. An inhibitory PN response was detected using the following two conditions: (i) amplitude criterion: odor-evoked neural firing rates (averaged over trials) do not exceed 2 standard deviations of baseline activity (2 s pre-stimulus activity averaged over trials) in any time bin during odor presentation. Further the mean firing rate during the entire stimulus...
duration must be lower than the mean baseline activity; (ii) reliability criterion: the amplitude criterion must be met in 50% of trials.

**Spike Counts Comparison of Individual ORNs and PNs:** All analyses involving spike counts comparison of individual neurons were done using cells with an ‘excitatory response’, as defined above. The amplitude and reliability criteria have to be met in at least one stimulus presentation condition for the cell to be included in this analysis. Spike counts for cells satisfying both amplitude and reliability criteria were computed in a two second window following the odor onset presented either alone or in an overlapping sequence. Comparisons were made between spike counts across conditions for each PN using one-way ANOVA at $P < 0.05$. Bonferroni correction was applied to account for multiple comparisons.

**Neural Synchrony Measure:** Spike trains of projection neurons were segmented into non-overlapping time bins (10 ms in Fig. 4.1e). Spike counts within each time bin was summed across neurons and averaged over trials. A three-point average zero-phase digital filter was used to smooth the raw spike counts computed.

**Correlation of Ensemble PN Response Profiles:** For each PN and for each trial, spike counts were summed over the entire 4 s odor presentation period following the solitary introduction of an odorant or following the introduction of the second stimulus in the overlapping sequences. For a particular trial, this resulted in $n$ spike counts for $n$ PNs in the dataset. The different correlation values shown in Fig. 4.1g were computed as follows:

1. PN spike count profiles obtained during different trials (solitary introductions) were correlated in pairwise fashion (inter-trial correlation),
2. correlations between trial-averaged response profiles generated during solitary vs. overlapping condition (inter-condition correlation),

3. correlations between trial-averaged response profiles generated during solitary introductions of two different odorants presented in an overlapping sequence (inter-odor correlation),

A two-way ANOVA with bonferroni correction was used for the statistical testing. No significant difference was observed between the inter-trial group and inter-condition group ($P = 0.12$). However, inter-odor correlation values were significantly lower than the inter-trial group ($P = 1.53 \times 10^{-5}$) and the inter-condition group ($P = 7.25 \times 10^{-4}$). Moreover, no significant differences were discerned between different odor groups ($P = 0.58$).

**Percentage of Co-activation:** For the analysis shown in Fig. 4.4d, percentage of PNs that were activated by both odorants in an odor sequence was calculated using the following equation:

$$
    \text{co-activation} = \left( \frac{\# \text{ of PNs that responded to solitary introductions of both odorants}}{\# \text{ of PNs that responded to the solitary introduction of the second odorant}} \right)
$$

**Neural Response Latency:** Response latency of a neuron was conservatively defined as the time taken for the stimulus-evoked activity to reach 20% of the peak firing rate (bin size = 50 ms). To account for variations in the spontaneous firing rates that might otherwise influence the response latency calculations, the stimulus-evoked firing rate responses were standardized first by subtracting the mean spontaneous activity observed during a 2 s pre-stimulus period. The same criterion was also used for determining the latency of a palp-opening response in our behavioral assay.
Local Field Potential (LFP) Analysis: LFP signals were acquired at 15 kHz and filtered using an analog filter (3-1,000 Hz). For the analysis shown in Fig. 4.9, the raw data was subsequently re-sampled at 1 kHz and digitally filtered (5-55 Hz, 2nd-order Butterworth). Spectrograms were computed using a 500 ms sliding Hamming window with 90% overlap and averaged across experiments with 10 trials each. To allow comparison, power spectra computed for each trial were normalized by the maximum value in that trial. Cross-correlograms between signals simultaneously acquired from two electrodes were calculated using a 250 ms non-overlapping time windows and averaged across 10 trials.

For LFP analysis shown in Fig. 5.2a-c, raw extracellular waveforms were first filtered (5-55 Hz, 2nd-order Butterworth). A fast Fourier transform was used to compute the power spectrograms of the filtered extracellular signals during two separate epochs (0 - 500 ms and 2 - 4 s, after odor onset). The first time segment (0-500 ms) showed that the power of the spectrum peaked at around 20 Hz (fast; Fig. 5.2b top panel), while the later epoch (2 - 4 s) revealed the power of the spectrum peaked around 10 Hz (slow; Fig. 5.2b bottom panel). To allow comparison (Fig. 5.2c), power spectra computed for each trial were normalized by its mean power that was averaged within a 20 Hz band around the spectrum center (indicated by the dash lines in Fig. 5.2b). Repeated measures one-way ANOVA was used as the statistical testing to detect significant increase or decrease in standardized power.

Dimensionality Reduction Analysis: For this analysis, firstly the ensemble PN responses were arranged as time-series data of n-dimensions (n, number of neurons recorded) and m-steps (number of 50 ms time bins; m = 80). For the analysis shown in Fig. 3.4, and 3.10a, only the 4 s of PN activity during the solitary odor or foreground stimulus presentations were used for this analysis and dimensionality reduction was performed using locally linear embedding (LLE)
technique[108]. In Fig. 4.5a, the 4 s of PN activity during the solitary odor and the neural responses following the introduction of the second odorant in the overlapping sequence were used for this analysis and dimensionality reduction was performed using linear principal component analysis (PCA). The low-dimensional points were connected in a temporal order to visualize neural response trajectories to different stimuli. Plots shown were generated after smoothing with a 3-point running average low-pass filter. Note that for LLE analysis, qualitatively similar trajectories were generated for a wide range of neighborhood values (k = 10 to 35). The plots shown in Fig. 3.4 were generated with k = 15, 13, 16, 15, 33, and 11, respectively, and k = 15 for all the results shown in Fig. 3.10a. Note that the LLE analysis results were similar to those obtained using the linear principal component analysis.

**Tensor-based Data Decomposition:** The data were first organized as a three-way array \( \mathbf{X} \) (neuron \( \times \) time \( \times \) trials). Trials corresponding to different odorants were concatenated along the trial dimension of the data cube. A parallel factory analysis (PARFAC) was used to directly decompose the 3-way data cubes (Fig. 2.3a)[109] as follows:

\[
\mathbf{X} = \sum_{f=1}^{F} \mathbf{a}_f \odot \mathbf{b}_f \odot \mathbf{c}_f + \mathbf{E} \tag{2.2}
\]

where \( F \) is a positive integer denoting the number of components extracted from three-way array \( \mathbf{X} \), and \( \mathbf{a}_f, \mathbf{b}_f \) and \( \mathbf{c}_f \) are loading vectors with \( \mathbf{a}_f \in \mathbb{R}^I, \mathbf{b}_f \in \mathbb{R}^J, \) and \( \mathbf{c}_f \in \mathbb{R}^K \) for \( f=1,\ldots,F \). \( \mathbf{E} \) is the residual array of the same size as \( \mathbf{X} \) which holds the residual variation not explained by the \( F \) components. The symbol "\( \odot \)" indicate vector outer product. Note that an N-way array is rank one if it can be written as the outer product of N vectors. Therefore, this approach decompose the three-way array into a sum of rank-one components. The tri-linear model is found using alternating least squares that minimized the mean-squared reconstruction error. As noted, the
number of factors is a free parameter that is determined using a core consistency diagnostics[110]. The core consistency metric measures the goodness of fit for the approximated dataset. For the datasets used in Chapter 5, the core consistency metric was above 80% for F<=3 but fell below 40% when $F=4$ (Fig. 5.3b). Therefore, a three factor decomposition was used for generating results shown in Fig. 5.3b, 5.4a,b.

Figure 2.3 Tensor-based data decomposition. (a) A three-dimension neural data array can be approximated as a sum of multiple rank-1 tensors that are obtained through outer product of three loading vectors $a_f$ (neuron dimension), $b_f$ (time dimension), and $c_f$ (trial dimension) (subscript $f$ ranges from 1 to the total number of factors). (b) The loading vectors are used to reconstruct the three-way data. This reconstructed data cube is unfolded and concatenated with respect to the trial dimension, to which the dimensionality reduction technique (e.g. PCA) can be applied for odor trajectory visualization.

**Trial-to-trial Odor Trajectory:** For this analysis, the dataset was first approximated by the outer product of the loading matrices that were obtained by the tensor decomposition, and then unfolded and concatenated along trial dimension (Fig. 2.3b). As a result, the ensemble projection neuron responses were organized as time series data of $n$ dimensions (where $n$ is number of neurons) and $m$ steps (the number of 50 ms time bins $\times$ the number of trials). Finally, principal component analysis (PCA) was applied to generate odor trajectories on a trial-to-trial basis. Note
that three PC axes capture 100% variance of the dataset, since a sum of 3 rank-one components is used to approximate the dataset.

**Classification Analysis of PN Ensemble Responses:** Ensemble PN spike counts were considered in a 50 ms non-overlapping time bin as a high-dimensional response vector. Response vectors obtained during solitary odor exposures were regarded as the desired reference templates to be pattern matched. Five trial-averaged reference templates were generated for each odor (4 s pulse duration). These reference templates represented the mean ensemble PN activity during the following temporal response segments: four one-second windows following odor onset (0–1 s, 1–2 s, 2–3 s, 3–4 s) and a 2 s window following odor offset.

**Figure 2.4 Classification analysis.** First, high-dimensional PN activity patterns obtained during pure odor presentations are used to construct reference template vectors for each background and foreground odor. Five trial-averaged reference templates are constructed for each odor that represent mean activity during the following time bins: 0 to 1 s, 1 to 2 s, 2 to 3 s, and 3 to 4 s following odor onset, and a 2 s window following stimulus termination. The classification analysis is restricted to quantify meaningful pattern-match using two parameters: angular tolerance and a detection threshold. Any trial not used to create the reference templates is regarded as a test trial. The odor-evoked activity patterns are categorized to the same odor class as their nearest reference template. Blue pixels indicate proximity to the background odor templates, and red pixels indicate similarity with foreground odor responses. Sub-threshold activities are identified using gray pixels, whereas, any PN activity patterns outside an angular distance tolerance limit are indicated using black pixels. This analysis is repeated for all time bins in a test trial.
For classifying trials that involved solitary odor presentations, we followed the leave-one-trial-out validation approach. In other words, nine trials were used as training trials for constructing the reference templates and the excluded trial became the test trial. This was repeated ten times such that each of the ten trials was made a test trial once. For overlapping conditions all ten trials were regarded as test vectors to be classified using templates obtained from solitary odor exposures. To identify meaningful response patterns, here two criteria were defined. First, there was a threshold length that must be exceeded by a vector to be considered as an odor-evoked response. The threshold was set as the mean length of pre-stimulus activity vectors + 2 standard deviations. Second, a tolerance threshold was defined that would restrict the classification analysis to include only those vectors that are within a certain angular distance (85° angular distance threshold used for all classification analyses) to any one of the desired response templates. Angular distances between a given test vector \((\mathbf{V}_t)\) and each reference vector \((\mathbf{V}_r)\) was computed as follows:

\[
\text{angular distance} = \cos^{-1}\left(\frac{\mathbf{V}_t \cdot \mathbf{V}_r}{|\mathbf{V}_t||\mathbf{V}_r|}\right)
\]  (2.3)

Only those test vectors that exceeded the detection threshold but were within the defined tolerance threshold were classified.

For the classification results used in linear regression analysis in Fig. 3.8e, pattern match with only the foreground odor reference templates were determined and plotted. It is found that the result was robust (i.e. \(R^2 > 0.46; P < 0.05\)) for a wide range of angular tolerance threshold values \((65^\circ \leq \theta \leq 85^\circ)\). Correlation results shown in Fig. 3.8e were determined with \(\theta=75^\circ\). While for the classification analysis in Fig. 4.5b, only those test vectors that were within the defined tolerance threshold were classified. Note that no standard deviation test was done to limit
the classification analysis to odor onsets and offsets when comparatively stronger responses were observed.

**Significance Analysis:** Two metrics were defined to assess the significance of obtained classification results. First, baseline misclassification rates were determined as the percentage of time bins (averaged across trials) during the 2 s pre-stimulus baseline period that were classified (Fig. 2.5a). The probability of classification during pre-stimulus periods was less than 5%. Second, vector templates experimentally obtained for all six odor pairs in chapter 3 were used to ascertain classification rates when a large number of randomly generated unit-vectors (100,000 vectors) were made as the test set. These 100,000 vectors were distributed such that their mean was chosen to be at 90° from the mean reference vectors (during odor presentations). Individual random vectors were obtained by adding a unit standard deviation noise to the mean vector. Only ~5% of the generated random vectors were within the tolerance limit by chance (Fig. 2.5b). All other vectors exceeded the tolerance threshold and were not classified into any odor category (Fig. 2.5c).
Figure 2.5 Significance analysis. (a) Percentage of time bins during pre-stimulus periods that were classified is less than 3% for all odor pairs. (b) Histograms revealing the distribution of angular distances between individual test patterns and their closest reference templates are shown. Same coloring scheme as used in panel a. Only vectors exceeding standard deviation test were included in this analysis. Black bars represent angular distance greater than 85°. The mean angular distance was between 68.65°–71.79°. (c) Classification of random vectors using reference vector templates obtained for each odor pair. Less than 5% of the random vectors were within the tolerance limit by chance. All other vectors exceeded the tolerance threshold and were not classified into any odor category.

Neighboring-trial Similarity Analysis: To quantify how neural response patterns change over trials we adapted a correlation analysis first proposed in[111]. In this approach, spike trains were first binned using 50 ms non-overlapping timebins. The spike counts during the entire stimulus 4 s duration generated an 80 dimensional time series for a single trial. To characterize the change in stimulus-evoked responses that happen across trial, we computed the correlation coefficient between two time-binned spike trains, one is from a given trial and the other is the average of its neighboring two trials (Fig. 2.6a).
\[ Correlation = \frac{(x_i - \bar{x}_i)(x_{avg} - \bar{x}_{avg})}{\sigma_i \sigma_{avg}} \]  

(2.4)

where \( x_i \) indicates the spike train vector of trial \( i \), and \( x_{avg} \) denotes the average of two neighboring spike train vectors of trial \( i-1 \) and \( i+1 \). \( \bar{x} \) represents the mean response within a vector, and \( \sigma \) denotes the standard deviation. Note that for the first and the last trial, we used two immediately following and preceding trials respectively.

This correlation metric resulted in various profiles for a total of 644 neuron-stimulus pairs. To further categorize the systematic pattern, I first smoothed those correlation profiles with a five-point moving average filter. Subsequently, I performed hierarchical clustering. For this clustering analysis, a correlation distance was used to calculate the pairwise distance between two response profiles. The cluster was formed such that the furthest pairwise distance between any two samples assigned to an individual cluster was minimized. The optimum number of clusters required to represent the entire dataset was chosen based on the mean-squared error (Fig. 2.6b). Using this approach, I was able to identify distinct trial-to-trial response change patterns (Fig. 5.1b). A schematic of this analysis is shown in Fig. 2.6.
**Figure 2.6 Neighboring-trial response similarity analysis** (a) Schematics of neighboring-trial response similarity analysis. Step 1: raster plot of a single PN response to a 4 s stimulus over 25 repeated trials. Each tick mark indicates a spike. Color box indicates odor presentation. Step 2: the spike trains for each individual trial were binned using 50 ms non-overlapping timebins. The spike counts during the entire stimulus 4 s duration generated an 80 dimensional time series for a single trial. The trial number is coded by a color gradient. Step 3: Correlation coefficient were calculated between neural activity of a given trial and the average of its neighbors. Step 4: the calculated correlation coefficients are plotted as a function of trial number. (b) Mean squared error is plotted as a function of the number of the clusters. The optimal number of cluster is determined as the elbow of the curve.
**Computational Model:** For the analysis shown in **Fig. 4.8**, an ensemble of 100 projection neurons were simulated where each individual PN’s firing activity was modeled as an alpha function:

\[
Neuron \ response = Amplitude \times t \times e^{-t^{1.25}/\tau}
\]  

(2.5)

where \( Amplitude \) is the peak response amplitude, \( t \) represents the time, and \( \tau \) is the response time constant. The response amplitude for each cell was generated randomly from an exponential distribution with mean value of 10 Hz. The time constant for each cell was chosen randomly from a uniform distribution between 0 to 1 s. Ensemble responses to two different odorants (odor-1 and odor-2) were simulated by using two different sets of randomly chosen \( Amplitude \) and \( \tau \) values. Furthermore, the model was experimentally constrained as follows: 65% neurons were activated by odor-1 (corresponding to hexanol), 50% were activated by odor-2 (corresponding to 2-octanol), and the degree of response overlap was constrained by our real data (see **Fig. 4.4d**)

To simulate jittered versions of each odor, response delays were bootstrapped from the time-to-peak distributions (**Fig. 4.2**) derived from solitary and overlapping stimulus presentations of hex(hex) and 2oct(hex) odor pairs (for odor-1 and odor-2, respectively).

A MATLAB implementation of this model can be accessed at http://labs.seas.wustl.edu/bme/raman/publications/ncomm/ncmodel.m

**Responsive Kenyon cell categorization:** A KC was considered responsive to an odor stimulus if it responded with at least one spike during the odor presentation period in four or more trials (out of the total ten trials).
2.3 Behavioral Experiments and Analysis

**Palp-opening Response Behavioral Assay:** Behavioral experiments were carried out by adapting a protocol described in an earlier work[92]. Adult locusts of either sex were starved for 24 h before experiments. Locusts were immobilized within a plastic tube such that only antenna and mouthparts were freely movable. Both compound eyes were closed using black electrical tape to reduce influence of visual cues on behavior and to reduce spontaneous activity. For all experiments, we used the following odorants as the conditioned stimulus (CS): hexanol, isoamyl acetate and citral. Wheat grass was used as the unconditioned stimulus (US). The odor delivery setup was identical to our electrophysiology experiments. A video camera (Microsoft webcam) was used to capture the behavioral response of locusts during training and test trials and analyzed in a double-blind manner. A custom written Labview program controlled both the odor delivery and video data acquisition and thereby ensured a tight time match between them. An LED was used to signal the onset of the odor and the duration of odor delivery in the video clip.

Six trials were used to train each locust to associate the conditioning stimulus with the food reward. During each training trial, a 10 s CS was presented first, followed by a grass reward (US) that was offered 4 s following the CS onset. The inter-trial interval was set to 10 min. To exclude any preconditioning to the CS, locusts that responded to the CS in the first training trial were eliminated from further experiments (< 15%). Only those locusts that accepted the reward in at least 4 out of 6 training trials were used for testing (> 80%).

Selective opening of maxillary palps to the CS presentation was used as an indicator of the acquired memory. The palp-opening response (POR) was considered positive if the locusts opened one or both of their maxillary palps crossing an imaginary response threshold (red dashed line in Fig. 3.8a) formed by the lateral groove of the labrum at least once during odor (4 s)
presentation. In Chapter 4, the POR analysis was improved with a finer temporal resolution. To precisely track the palp movements, a small amount of zero-volatile-organic-chemical green paint (Valspar Ultra) was applied to the distal segments of both the maxillary palps covering approximately 1 mm of the length of the palp. The painting of palps was done approximately an hour before the experiments for the locusts to become acclimatized with their coated palps and for the paint to dry. The distance between two maxillary palps was used as a metric to quantify the POR result (Fig. 2.7a-c).

We first assessed whether the associative learning of CS established during training was retained afterwards, and then performed four successive retention tests at 10 min, 40 min, 70 min, and 100 min after the last training trial (Fig. 3.8b). In each block of these retention tests, both trained (iaa) and untrained odor (bzald) were presented with 10 min interval without any reward. It is shown that the conditioned locusts had a significantly higher POR to trained odor than untrained odor (** $P = 1.22 \times 10^{-4}$, $6.10 \times 10^{-5}$, $7.63 \times 10^{-5}$, $3.05 \times 10^{-5}$; McNemar’s exact test, $n = 28$ locusts). Relative percentages of locusts that showed POR to the trained and the untrained odor across multiple tests remained consistent (Cochran’s Q test; for CS: $Q = 0.67$, df = 3, $P = 0.87$; for untrained odor: $Q = 2.2$, df = 3, $P = 0.53$).

Finally, we assessed whether locusts that exhibited selective POR to the CS were able to recognize the trained odor when presented solitarily or in an overlapping sequence. The stimulation protocol and the odor pairs used were identical to that used in our electrophysiology experiments. A total of four consecutive retention tests were performed at 10 min, 40 min, 70 min, and 100 min following the last training trial. These test trials comprised of the following set of stimuli presented in a random order: (a) the conditioned odor, (b) an untrained odor, and (c) the conditioned odor introductions during different dynamic epoch of background odor activity.
**Palp-tracking Algorithm:** To develop a quantitative, fine-grained behavioral analysis, the locust palp movements were tracked using a custom made Matlab program offline. Briefly, each video file was converted into a series of RGB color frames. After subtracting the grayscale image from the green channel of each frame, a 2-D Gaussian filter (10 pixel by 10 pixel; s.d. 10 pixels) was applied to emphasize the intensity of the green palps. The adjusted image was subsequently thresholded and filtered to generate a final image that contained only the painted segments of the maxillary palps. The centroids of the palps were tracked in all frames. One or both the palps were sometimes blocked from view due to movements of the antennae or poor video focus on the palps. In such cases, the palp positions were estimated from the previous frame. The precision of this palp tracking approach was subsequently visually inspected and validated on every single video. Four out of 75 locusts whose palps couldn’t be tracked in this automated fashion were eliminated from the dataset. Even if the tracking failed in only one test trial, all data from those locusts were removed from further analysis.

**Analysis of Behavioral Data:** There was very little palp activity during the pre-stimulus baseline periods on most trials. Therefore, even a small movement of palps that exceeded 20 pixels distance during single odor presentation was counted as response to the presented odorant. Distances between two palps were smoothed using a three-point zero-phase digital filter. The behavioral response latency was defined as the time when the POR distance reached 20% of its maximum value during trained odor presentation. The resting palp positions were either fully closed or slightly open. To remove this variability in baseline palp separation, I calculated the resting distance between the palps 10 s before the onset of the odorant and subtracted this distance from all other calculated distances.
**Pairwise Behavioral Response Correlations:** Cross correlations between palp movements of different locusts were calculated by firstly segmenting the entire behavioral response into overlapping 500 ms time segments (80% overlap between consecutive segments). A small amount of Gaussian noise (zero mean and 0.01 s.d.) was added to the calculated palp distances to eliminate infinite correlation values during time periods when the palps were held still. Note that this was the measure of predictability used in the analysis reported in Fig. 4.7b.

![Figure 2.7 Behavioral POR retention tests.](image)

**Figure 2.7 Behavioral POR retention tests.** (a) A snapshot of a video for the behavior experiment. Before the odor is delivered, the LED light is off and the maximally palp is kept closed, shown as left panel. A typical palp opening response can be observed when the odor comes that is indicated by the LED light on. The non-odorous green paint on palps facilitates precise tracking of their movement. (b) The palp positions are tracked over time in a 2 dimensional space by an image processing algorithm. (c) The palp opening response (POR) to the conditioned stimulus is quantified as the distance between palps over time.
**T-maze Assay:** A T-maze arena was designed with the following dimensions: 20 cm wide, 48 cm long, and 22 cm high (design adapted from an earlier work[92]; **Fig. 2.8**). An elevated T-bar (11 cm off the ground) was positioned centrally in this arena. The T-bar comprised of two wooden rods measuring 44 cm and 7 cm in length. The shorter side-arm split the longer rod into two arms of equal length. Locusts were kept restrained in a custom-designed holder that was located in the shorter side-arm of the T-bar. Two odor delivery ports (each 0.5 cm in diameter) were located on the sidewalls in line with the two arms of the T-bar. An 8 cm by 8 cm square vent right behind the locust holder housed a 5V exhaust fan. The exhaust fan ensured that there was a stable airflow inside the maze. The flow patterns were visually confirmed with titanium tetrachloride fumes. A transparent plexiglass lid was used to prevent locusts from escaping from the arena. A camera was used to record the movements of the locusts within the T-maze.

**Figure 2.8 Behavioral T-maze assay.** A schematic of the T-maze assay is shown. Locusts are restrained in a custom-designed holder and released just before the odor delivery. A test odor and the control (mineral oil) are simultaneously presented at the two odor delivery ports. An exhaust fan at the center of the maze ensured that there is a stable airflow inside the maze (flow patterns are confirmed with titanium tetrachloride). Each locust is given 4 min to make a decision: i.e. select a T-maze arm, reach and touch the sidewall at the end of the selected arm with its leg or antenna.
Locusts were selected using similar procedures as used in our electrophysiology experiments. Locusts were starved for 24 hours before the experiments. Locusts were initially kept restrained in a custom-designed locust holder and released just before the odor delivery. A test odor (1% concentration v/v) and the control (mineral oil) were simultaneously presented at the two odor delivery ports. A 4 min odor pulse was delivered by injecting a constant volume (0.1 L/min) of the static headspace above the odorants/mineral oil into a desiccated air stream (0.2 L/min). To prevent depletion of headspace in odor bottles, stimulus delivery was programmed to seamlessly switch between two equivalent odor bottles every 12 s.

Locusts were given 4 min to make a decision: i.e. select a T-maze arm, reach and touch the sidewall at the end of the selected arm with its leg or antenna. The overall locust preference in the T-maze was quantified as follows:

\[
\text{Preference Index} = \frac{\text{Number of locusts (towards odor)} - \text{Number of locusts (away from odor)}}{\text{Total number of locusts (towards + away)}}
\]  

Locusts that did not choose a T-maze arm or went off them were discarded from the final analysis (< 20% of the total locusts). Statistical comparisons were performed using exact binomial test with Bonferroni correction for multiple comparisons at a 0.05 confidence level.

Each locust used in our T-maze experiments was used only for one trial. The entire T-maze was cleaned with ethyl alcohol between trials to prevent any conspecific cue from the previous trial from influencing the overall results. Further, the test odor and the control odor ports were randomly chosen for each trial to prevent any directional bias from influencing our results.
Locusts were kept on a 12 h day – 12 h night cycle (7 am – 7 pm day). All behavioral experiments were performed between 9 am – 3 pm.

2.4 Justifications for Statistical Tests

All statistical significance testing done in the manuscript were two-sided. Bonferroni corrected $P$ values were used for all multiple comparisons. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications in the field[68, 69, 73, 75]. For behavioral experiments, the sample size was large enough to demonstrate statistical significance. Even in cases where statistical significance was achieved with a smaller sample size, $n = 20$ locusts were used.

For the paired t-tests, one-way ANOVA and two-way ANOVA, normality of the dataset was confirmed using the Jarque-Bera test. The equal variance assumption was examined using both Levene’s and Bartlett’s test. The equal variance assumption was met in ~70% of PNs studied (at $P = 0.01$ significance level). In addition, for two-way ANOVA tests, the observations within and between groups were assumed to be independent.

Levene’s test was used to assess the homogeneity of variance between time-to-peak-response distributions. No normality test was performed because this test is suitable for non-normally distributed data as well.

McNemar’s exact test was used for statistical comparison of POR of the same locust under different conditions (nominal data with matched pairs of subject). Cochran’s Q test was used to compare the performance of locusts in four back-to-back retention tests (Fig. 3.8b). It is an extension of McNemar's exact test for $k > 2$ experiments.
Exact binomial test was used to statistically compare the distribution of locusts that went towards or away from the odorant delivered (Fig. 3.9a). For these tests the sample sizes were small ($n = 20$), the trials were independent (different locusts were used for each trial) and probability of success was constant.

Wilcoxon signed-rank test is a non-parametric test for comparing the population median responses of matched samples. This test was used to detect whether a significant increase in palp-opening responses was elicited by the second stimulus in the sequence (Fig. 4.7a), and to assess whether the predictability of palp-opening responses on the same set of locusts differed depending on how the conditioned stimulus was presented (Fig. 4.7c).

The Wilcoxon rank-sum test was used to compare the median population response for non-paired samples. This test was used to determine whether the neural and behavioral response latency distributions between odorants, i.e. the comparison between hexanol and isoamyl acetate responses, were significantly different.

Linear regression analysis assumes that data can be fit using a straight line and sample points are independent of each other. Both of these assumptions were met in our analysis to detect the relationship between neural response overlaps and reduction in synchrony (Fig. 3.8e and 4.4e). Pearson’s correlation was used to determine the degree of linear relationship between neural synchrony and behavioral response predictability (Fig. 4.7b). A linear relationship between the variables examined was assumed.

Repeated measures one-way ANOVA was used to assess the significant changes of the mean LFP’s oscillatory powers over repeated trials (Fig. 5.2c). The measurement was repeated
on the same sets of the insects, and data in each group satisfied the normality (Jarque-Bera test) and equal variance assumption (Levene’s test).
Chapter 3: A Spatiotemporal Coding Mechanism for Background-Invariant Odor Recognition

3.1 Introduction

Sensory stimuli often evoke temporal patterns of spiking activity across a population of neurons in the early processing stages [62, 106, 112-121]. These neural responses are considered a ‘temporal code’ if they change on a timescale that is different than the stimulus variations that caused them and when they convey useful information about the stimulus [41]. A fundamental problem in sensory neuroscience is determining what stimulus-specific information is encoded by dynamic patterns of ensemble neural activity and whether this information is behaviorally relevant. Furthermore, since the same stimulus can be encountered in a variety of ways in natural environments, what attributes of the spatiotemporal population responses are invariant to any or all such variations?

The insect olfactory system is a widely used model for studying odor coding and behavior [50, 92, 122-124]. In this system, odorants are detected by olfactory receptor neurons (ORNs) in the antenna, which transduce chemical stimuli into trains of action potentials. The ORN signals are relayed downstream to the antennal lobe, where spatiotemporal patterns of activity across ensembles of projection neurons (PNs) represent odors. The odor-evoked PN responses are elaborate and change most rapidly following stimulus onset and offset (referred to as ‘on-transient’ and ‘off-transient’ responses, respectively). For lengthy odor presentations, the antennal lobe activity converges to a steady-state within ~ 1.5 s of stimulus onset[66, 67, 125].
These dynamic neural activity patterns are repeatable across trials, and contain information about odor identity and intensity [68, 69, 75].

The time-varying response patterns are maximally informative during transient phases of neural activity [66]. Odor representations are most distinct during these epochs and predominantly drive spiking activity in downstream neurons[66, 122]. Consistent with these physiological results, behavioral data indicate that animals are capable of discerning between ‘similar’ odors (a relatively difficult task), within a few hundred milliseconds of stimulus onset [70-73]. Taken together, these results suggest that initial segments of the on-transient response contribute significantly for encoding odor identity.

What then is the importance of neural activity that follows the initial response segment? In the olfactory system, even a very brief odor pulse (lasting a few hundred milliseconds) can generate an elaborate response that lasts for a few seconds. This prolonged stimulus-evoked activity appears to be more a liability than a feature, as it could potentially interfere with and corrupt the olfactory system’s response to other chemical cues in the environment. Two previous studies[57, 75, 76] that examined this issue reported results that are rather contrasting. While pulses of the same stimulus generated ensemble activity that was reliable and repeatable at the population level, overlapping pulses of different stimuli created spatiotemporal responses that often interfered with each other heavily, thereby making them unpredictable. In both cases, however, the activity was not reset to baseline levels before responses to the freshly introduced stimuli began.

The existence of hysteresis in the olfactory system[76] poses a conundrum, as it may hinder any coding scheme using dynamic patterns of neural activity. Yet, spatiotemporal patterns

3.2 Results

We sought to systematically perturb the spatiotemporal responses evoked by an olfactory stimulus. To achieve this objective, we exploited the physiological observation that odor-evoked activity in an ensemble of sensory neurons and their followers in the antennal lobe and mushroom body are in a highly dynamic state after odor onset (for ~1.5 s) and offset (~3 s) (referred to as ‘on-transient’ and ‘off-transient’ respectively; Fig 3.1a). Between these two transient phases, the odor-evoked responses converged to a steady-state activity. Therefore, we used sequences of stimuli, where the delay between the onset of the first (‘background’) and the second odor (‘foreground’) was varied. The foreground odor introductions were manipulated to occur either concurrently with the background (‘binary mixture’), or, during on-transients, steady-states, or off-transients of the background odor activity (Fig 3.1b). This odor stimulation protocol enabled us to probe if and when the on-going olfactory antennal lobe dynamics allow stable representation of a newer stimulus.

To understand general processing principles that govern how fresh stimulus introductions are tracked by the locust olfactory system, we identified a diverse set of six background–foreground odor combinations (Fig. 3.1c). Notably, the selected odor pairs included odorants that belong to the same functional group, different functional groups and a pair of complex blends. Note that this selected stimulus set also accounted for diversity with respect to vapor pressure and relative sensory input strengths as measured by the electroantennogram signals.
Figure 3.1 Neural dynamic states and a diverse set of odor combination. (a) Mean firing rates across olfactory receptor neurons (ORNs), projection neurons (PNs) and Kenyon cells (KCs) are shown as a function of time \((n = 238\) ORN-odor combinations; \(n = 1450\) PN-odor combinations; \(n = 198\) KC-odor combinations). The 4 s odor stimulation period is indicated as a gray bar along the x-axis. (b) A schematic representation of the stimulation protocol used (top to bottom): background alone stimulus (Back), foreground alone stimulus (Fore), simultaneous presentation of both the background and the foreground odor (Mix), foreground odor introductions during on-transient (OnTr), steady-state (Steady), and off-transient (OffTr) phases of background odor activity. (c) A diverse set of background–foreground odor combinations were chosen for the study, where the selected combinations are from either same or different functional group, elicit different EAG response strength, and form various vapor pressure levels.

<table>
<thead>
<tr>
<th>Odor pairs (Background–foreground)</th>
<th>Functional group</th>
<th>EAG response</th>
<th>Vapor pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-octanol – hexanol ((2\text{oct}–\text{hex}))</td>
<td>Same</td>
<td>(2\text{oct} &lt; \text{hex})</td>
<td>(2\text{oct} &lt; \text{hex})</td>
</tr>
<tr>
<td>Cyclo-hexanone – 2-heptanone ((\text{chex}–\text{2hep}))</td>
<td>Same</td>
<td>(\text{chex} = \text{2hep})</td>
<td>(\text{chex} &gt; \text{2hep})</td>
</tr>
<tr>
<td>Benzenaldehyde – isoamyl acetate ((\text{bzald}–\text{iaa}))</td>
<td>Different</td>
<td>(\text{bzald} &gt; \text{iaa})</td>
<td>(\text{bzald} &lt; \text{iaa})</td>
</tr>
<tr>
<td>Hexanal – hexanol ((\text{hxa}–\text{hax}))</td>
<td>Different</td>
<td>(\text{hxa} &lt; \text{hex})</td>
<td>(\text{hxa} &gt; \text{hex})</td>
</tr>
<tr>
<td>Geraniol – citral ((\text{ger}–\text{cit}))</td>
<td>Different</td>
<td>(\text{ger} &gt; \text{cit})</td>
<td>(\text{ger} &lt; \text{cit})</td>
</tr>
<tr>
<td>Mint – apple ((\text{mint}–\text{apple}))</td>
<td>Complex blend</td>
<td>(\text{mint} = \text{apple})</td>
<td>–</td>
</tr>
</tbody>
</table>

Vapor pressures are compared at 25°C.
3.2.1 Sensory Neuron Responses to Overlapping Stimuli

We found that response to the foreground odor introductions in a subset of ORNs remained unchanged regardless of whether or not they were preceded by a background stimulus (Fig. 3.2a, top row). Such reliable responses were commonly observed for those ORNs that responded only to the foreground odor. However, when an ORN responded to both stimuli presented in a sequence, or only to the background odor, the spiking activity elicited following foreground odor introductions became inconsistent across different conditions (Fig. 3.2a, bottom row). In some cases, even when the background stimulus did not elicit a response, the ORN spiking activity following foreground odor introductions became diminished due to the presence of the background stimulus (Fig. 3.2a; chex–2hep).

We found that when two odors were presented concurrently (i.e. the binary mixture case) their responses combined in simple ways (Fig. 3.2b). The binary mixture generally elicited a stronger response as it combined spiking activities of component stimuli. The influence of the background odor activity on the foreground odor response diminished as the delay between background and foreground odor onsets increased (Fig. 3.2c,d). Our results indicate that even though blends of the same two odors were presented, ORN responses can vary depending on the temporal overlap between the two stimuli.
Figure 3.2 Individual ORN responses with background history. (a) Representative raster plots of six ORNs are shown. Each raster plot encompasses six blocks of trials that correspond to the different stimulation conditions identified in Fig. 3.1b (in the same order). For each condition, responses in five trials are shown for assessing repeatability. Colored boxes specify whether the background and foreground odors were presented alone or in an overlapping fashion. A gray box is used to identify periods of stimulus overlaps. (b) Peristimulus-time histograms (PSTH) are shown for representative ORNs (encompassing all six odor-pairs) that reveal different ways in which foreground odor responses can change when presented following another stimulus. For each ORN, responses to background and foreground odors (component responses) are shown along with their response to one of the overlapping conditions. Red, blue and gray PSTH respectively indicate increase, decrease and no significant change in the foreground odor response (spike counts) when the same stimulus is presented in a sequence. The maximum firing rate (in Hz) is specified for each ORN. Each column corresponds to a specific sequence of an odor pair. (c) Comparison of mean ORN spike counts in a 2 s window following foreground odor onset. The X-axis corresponds to spike counts when the foreground odor is presented alone. The Y-axis corresponds to spike counts when the foreground odor is presented following a background stimulus. Mean ± s.e.m. over five trials is shown for all cells. Cells marked in red color indicate a significant increase in spike counts during the overlapping conditions and are therefore above the diagonal ($P < 0.05$, df = 4, 20; one-way ANOVA with Bonferroni correction for multiple comparisons; $n = 111$ ORNs were recorded from 27 locusts). Similarly, cells marked in blue indicate a significant decrease in spike counts, and those in gray indicate no significant change across the two conditions. (d) Total number of ORNs with a significant increase (red) or decrease (blue) in spike counts.

These observations raise the following important question: how similar are the sensory inputs generated by the same stimulus under different background and no-background conditions? To understand this we compared the mean number of spikes elicited in a two second window following the foreground odor onset presented either alone, or during different dynamic phases of the background odor activity (Fig. 3.2c). We found that the mean spike count changed significantly in a substantial number of ORNs (off-diagonal cells in Fig. 3.2c; $P < 0.05$, df = 4, 20; one-way ANOVA with Bonferroni correction for multiple comparisons; see Online Methods). Compared to the no background case, in most ORNs, spike counts increased during concurrent, on-transient and steady-state introductions of the foreground stimulus but reduced during off-transient introductions (Fig. 3.2d). Hence, our results show that the same stimulus can generate variable activity across an ensemble of ORNs when presented simultaneously or following a background odor.
3.2.2 Projection Neuron Responses Vary with Stimulus History

Next, we examined whether the variability observed in sensory neuron responses to the same stimulus affects its subsequent processing in the downstream antennal lobe circuits. We made extracellular recordings to monitor the odor-evoked activity in PNs that receive ORN inputs (\(n = 725\) PNs from 70 locusts were recorded; includes all six odor pairs). We found that PN responses to odor mixtures were more complex. For example, even when both component odors individually increased spiking activity in a PN during odor presentation, their mixture could still reduce activity to below spontaneous levels (Fig. 3.3a; PN5). Furthermore, our results indicate that even if responses to the individual odors were known, for a substantial number of PNs, it would still not be possible to predict their responses to the same pair of stimuli when presented as a binary mixture or in a sequence (Fig. 3.3a).

We found nearly 70\% of all recorded PNs showed substantial deviation from baseline activity following either background or foreground odor introductions (\(n = 502\) of the 725 PNs recorded; see Methods of Chapter 2). The response evoked by the foreground stimulus in nearly two-thirds of these PNs varied depending on the presence or the absence of a background odor. Interference was observed in overlapping but non-identical subsets of PNs for binary mixture, on-transient, steady-state and off-transient introductions (Fig. 3.3a). For all odor pairs used, we also found a large, non-overlapping subset of PNs that showed reliable responses to the freshly introduced odor under all conditions (\(n = 181\) of the 502 PNs). Overall, our results suggest that it would not be possible to predict whether and how a PN’s response to a foreground odor would change with stimulus history.

To systematically quantify these results, we again compared the mean spike counts elicited by the foreground odor presented either alone or following a background odor (Fig. 3.3b;
P < 0.05, df = 4, 45; one-way ANOVA with Bonferroni correction for multiple comparisons). In contrast with the ORN results, the PN activity in general reduced during concurrent, on-transient and steady-state introductions for most odor pairs (Fig. 3.3b). Notably, increase in odor-evoked spiking activity was commonly observed during off-transient introductions. Thus, when encountering a sequence of odorants, PN responses and ORN spiking activity appeared to have an inverse relationship (Fig. 3.2d and 3.3c). More importantly, these results clearly show that odor-evoked response patterns in individual PNs to a stimulus can change unpredictably in the presence of a background odor.
Figure 3.3 Individual PN responses with background history. (a) Representative PN rasters are shown for all six odor pairs (rows) for different background-foreground sequences (columns). The selected cells clearly reveal that individual PN responses receive considerable interference due to on-going background odor activity. (b) Comparison of mean PN spike counts in a 2 s window following foreground odor onsets. The X-axis corresponds to spike counts when the foreground odor is presented alone. The Y-axis corresponds to spike counts when the foreground odor is presented following a background stimulus. Mean ± s.e.m. over ten trials is shown for all cells. The same analysis and color convention as used in Fig. 2c ($P < 0.05$, df = 4, 45; one–way ANOVA with Bonferroni correction for multiple comparisons; $n = 502$ PNs were recorded from 70 locusts). (c) Total number of PNs with a significant increase (red) or decrease (blue) in spike counts.

3.2.3 Dynamic Transitions Reshape PN Ensemble Activity

How stable are neural representations for odors distributed across an ensemble of PNs? To understand this issue and to visualize how the PN activity was dynamically reorganized following fresher stimulus introduction, we performed a dimensionality reduction analysis[69, 75] (Fig. 3.4; see Methods in Chapter 2). For this analysis, we arranged the ensemble of $n$ PN responses as $n$-dimensional time-series data (number of neurons recorded; $n = 116$ for 2oct–hex, $n = 104$ for chex–2hep, etc.) over 80 non-overlapping time bins (i.e. 4 s odor pulse duration). The time-varying PN responses were then projected onto three dimensions using a non-linear technique that generated a topology-preserving approximation of the original dataset (locally linear embedding[108]; LLE; similar plots using a linear principal component analysis are shown in Fig. 3.5). The low-dimensional points were connected in a temporal order to visualize the neural response trajectories generated by different stimuli.
Figure 3.4 Ensemble PN activities with background history. (a–f) Odor-evoked PN response trajectories are shown for all six background–foreground odor combinations. Each row represents trajectories corresponding to one particular odor-pair and each column represents one particular sequence of background–foreground odors. Trajectories show the mean PN ensemble response over time along three locally linear embedding axes (LLE). Numbers on the trajectory show time since odor onset in
seconds. B represents baseline activity before odor stimulation. Response trajectories were computed using spike counts in 50 ms non-overlapping time bins for the 4 s of odor stimulation (80 time slice data points per stimulus). Odor trajectories are shown for the following three conditions in the leftmost column: background odor (blue), foreground odor (red) and their binary mixture (green). Each stimulus generates a unique closed loop trajectory after dimensionality reduction. The red, green and blue trajectories are re-plotted in all other columns along with the black trajectory that traces the neural activity following foreground introductions during on-transient (second column), steady-state (third column), and off-transient (fourth column) background odor activity. (n = number of PNs).

We found that each odorant generated a unique, closed loop PN response trajectory that returned towards baseline levels within 2 s of odor onset. The binary mixture of two odors generated response trajectories that can be categorized into the following three cases: (a) combined contributions of both components and therefore occupied a region (or subspace) between the two component odors (e.g. 2oct–hex, chex–2hep and bzald–iaa), (b) dominated by one component and therefore remained closer to the stronger component trajectory (hxa–hex and ger–cit), and (c) responses evolved over time such that the mixture trajectory moved from one component to the other (mint–apple).

We found that introduction of a foreground stimulus in any of the three different dynamic states of background activity rapidly remapped the antennal lobe activity (black trajectories in Fig. 3.4). In 5 out of 6 odor pairs tested (except ger–cit), the introduction of the foreground odor during the on-transient phase of the background stimulus caused the PN response trajectory to transition towards the foreground odor response without returning to baseline activity. Foreground odor introductions during steady-state background activity generated response trajectories that evolved from close to baseline responses and in a direction aligned with the foreground odor response. The off-transient introductions of the foreground odors also resulted in response trajectories that began close to the baseline response, but for some odor pairs, generated trajectories that were less overlapping (Fig. 3.4, fourth column; 2oct–hex, ger–cit and
mint–apple). More importantly, in all cases, the dynamic reorganization of ensemble activity over time resulted only in partial pattern-matches with neural representations evoked by the same foreground stimulus when presented alone.
Figure 3.5 Visualization of ensemble PN responses using linear principal component analysis (PCA). PCA trajectories to all six odor pairs are shown. Same convention as that used in Fig. 3.4. The same sets of PNs used in Fig. 3.4 were used for generating the PCA plots.
3.2.4 Piecewise Classification Allows Robust Odor Recognition

The dimensionality reduction analysis qualitatively revealed similarities between spatiotemporal ensemble responses for pure odors and their overlapping sequences. How significant are these observed response overlaps? To understand this, we performed a quantitative, trial-by-trial classification analysis (see Fig. 2.4 in Chapter 2). We considered the high-dimensional ensemble PN firing patterns elicited during solitary foreground and background odor exposures as the desired reference templates to be pattern matched. Five trial-averaged reference templates were generated for each odor to represent the mean ensemble PN activity in epochs during and immediately after stimulus presentations. Ensemble PN spike counts in trials not used to create reference templates were regarded as test response patterns to be categorized. Each test pattern was subsequently assigned to the category of its best matching reference template (i.e. smallest angular distance; background – blue pixel, foreground – red pixel). This classification analysis was done on a bin-by-bin, trial-by-trial basis for all six odor pairs (Fig. 3.6).

We found that binary mixture responses that combined contributions from both components (2oct–hex, chex–2hep and bzald–iaa), had significant classification probabilities for both background and foreground odors (Fig. 3.6a–c; refer to Methods in Chapter 2 for significance analysis). In comparison, when one component dominated the mixture response (hxa–hex and ger–cit), the classification analysis also revealed that those responses were more likely to be categorized with only one odor (Fig. 3.6d,e). Classification of the mint–apple mixture confirmed impressions from our trajectory analysis that the response vectors were initially similar to the mint odorant but evolved over time to pattern match with the apple response (Fig. 3.6f).
Figure 3.6 Classification results for all background–foreground odor pairs. Each panel reveals six classification blocks corresponding to the stimulation conditions identified in Fig. 3.1b. Odor identities (blue – background, red – foreground) and presentation durations are shown below each block. A leave-one-trial-out scheme was followed for classification of pure foreground and background odor trials. Right panels show classification probabilities for each time bin (i.e. percentage of blue or red pixels for a given time bin) during the first 2 s following foreground odor onsets (and following background odor onset when presented alone; first block). $n =$ number of PNs.
Furthermore, similar to our trajectory analysis, dynamic introductions of foreground odors atop background odors were detected and recognized during all three dynamic states for all odor pairs except ger–cit. For the on-transient introductions, the classification probabilities changed in a contiguous manner indicating that the response patterns were initially more similar to the background reference templates but subsequently gained similarity with the foreground odor response. Whereas, for the steady-state and off-transient introductions, a brief return to sub-detection threshold response allowed the classification periods following the two stimulus onsets to become temporally decoupled (Fig. 3.6). In sum, the classification results matched qualitative observations from our dimensionality reduction analysis.

Hence, our results suggest that the PN population response, when processed in a piecewise manner, can allow robust recognition of most odors independent of their backgrounds.

3.2.5 Kenyon Cells Robustly Respond to a Fresher Stimulus

Do the downstream centers that receive input from the antennal lobe take advantage of the dynamic processing of olfactory signals? To examine this we first studied the overall peri-stimulus time histograms (PSTHs) combining all Kenyon cells’ (KCs) activity to each odor used in our study. Consistent with previous results [18, 122], we found that KCs in the mushroom body responded with temporally sparse activity predominantly at odor onset and offset. During the middle portion of a lengthy odor presentation, i.e. during steady-state antennal lobe activity, we found that KC firings returned back to baseline levels for all odors tested (Fig. 3.7). This result suggests that firing in these cells is limited to those temporal epochs when the afferent activity was in a dynamic phase with higher firing rates.
Figure 3.7 KCs are sensitive to partial pattern matches in the AL activity. Raster plots showing responses of twelve different KCs to each background-foreground odor combination. Each KC raster plot is arranged following the scheme described in Fig. 3.1b.

We found that a few KCs were less selective and responded to both background and foreground odors (Fig. 3.7; 44 of 99 KCs; e.g. KC1 and KC3; see Methods of Chapter 2). Such responses, however, were less common for those odor pairs not belonging to the same functional group (15 of 55 KCs; e.g. KC5 and KC10). A few KCs responded to all introductions of a particular foreground odor suggesting that any partial overlap in the projection neuron ensemble responses was sufficient to drive spiking activity in these KCs (e.g. KC2, KC5 and KC7). In sum, our results indicate that KC responses are consistent with the information content of the PN
ensemble responses and a subset of KCs can respond to odors independent of the stimulus presented before them.

### 3.2.6 Predicting Behavior from Physiology Results

Can overlaps observed in the antennal lobe ensemble neural activity also predict behavioral recognition of odors? To investigate this issue, we assessed the recognition performance of locusts in an appetitive-conditioning paradigm[92] (Fig. 3.8a). In this assay, each locust was trained to associate a conditioned stimulus (CS; hex or iaa or cit) with a grass reward (unconditioned stimulus) that followed the CS presentations (see Methods in Chapter 2). After six training trials, the behavioral response of each trained locust was evaluated in an unrewarded test phase. Selective opening of maxillary palps (sensory appendages close to the mouth area) to CS presentations during test trials was used as an indicator of the acquired memory.

We found that the performance of trained locusts in unrewarded test trials remained consistent even when assessed using multiple test trials (Fig. 3.8b). Taking advantage of this result, we tested each trained locust by presenting the following set of stimuli in a random order: (a) the conditioned odor (hex or iaa or cit), (b) an untrained odor (2oct or bzald or ger), and (c) the conditioned stimulus presented atop a background odor in each of the three dynamical states (Fig. 3.1b).
Figure 3.8 Behavioral performance correlates with classification analysis results. (a) A schematic of locust palp opening response (POR). Dotted red line indicates the POR threshold used to determine a response. (b) PORs are shown for four consecutive blocks of unrewarded test trials with 30 min interval between two consecutive blocks. Conditioned locusts had a significantly higher POR to the trained odor (iaa) during all four test trials (**P = 1.22×10⁻⁴, 6.10×10⁻⁵, 7.63×10⁻⁵, 3.05×10⁻⁵; McNemar’s exact test, n = 28 locusts). The frequency of POR observed for trained and untrained odor remained consistent across the four consecutive test blocks (Cochran’s Q test; for CST: Q = 0.67, df = 3, P = 0.87; for untrained odor: Q = 2.2, df = 3, P = 0.53). (c) Bar graph summarizes PORs of n = 50 locusts during the testing phase. Note that the response to the CS (hex) was significantly higher than that observed during untrained odor (2oct) exposures (**P = 0.0052; McNemar’s exact test with Bonferroni corrected P values for multiple comparisons; n = 50 locusts). (d) Similar plots showing POR for bzald–iaa odor combination (n = 44 locusts). The POR to the CS (iaa) was significantly higher than that observed during untrained odor (bzald) exposures (**P = 8.64×10⁻⁷; McNemar’s exact test with Bonferroni correction; n = 44 locusts). (e) POR probabilities are plotted against the classification probabilities obtained from our quantitative classification analysis. A regression analysis (dashed line) revealed that the correlation between our physiology results and behavioral data was significant (R² = 0.6982, P = 0.0026; df = 8; n = 10 data points).

Amongst the three CS employed in our behavioral experiments, only hex and iaa resulted in effective associative learning (Fig. 3.8c,d). For these odors, nearly 70% of the locusts responded to the CS by opening their maxillary palps in anticipation of the reward. The palp-opening response (POR) to the CS presentations was significantly greater than the POR to an
untrained odor ($P = 0.0052$ for hex–2oct and $P = 8.64 \times 10^{-7}$ for iaa–bzald; McNemar’s exact tests Bonferroni corrected for multiple comparisons). We found that locusts rapidly responded to the conditioned odor with a median response latency of 0.58 s (i.e. onset of a visually detectable movement of palps). Expectedly, more locusts responded to an untrained odor (2oct) that belonged to the same functional group as the CS (hex).

How well do trained locusts recognize CS introductions that followed the untrained odor with varying latencies? Two orthogonal predictions can be made from our physiology data. If any pattern match in spatiotemporal responses were sufficient for recognition then we would expect both hex and iaa to be correctly recognized even when following another stimulus. However, if a precise match between odor-evoked activities was required then none of those introductions should elicit a behavioral response. We found that trained locusts were able to show PORs to conditioned odors irrespective of how they were introduced (Fig. 3.8c,d).

Next, we examined whether pattern matches in ensemble projection neuron responses predict locust POR in the behavioral assay? To determine this, for both conditioned odors (hex and iaa), we plotted the classification probability for each stimulus used during the testing phase against their palp opening response probability (Fig. 3.8e). For this analysis, PN firing pattern similarities were computed only with respect to the foreground odor response templates (see Methods of Chapter 2). As can be noted, a significant correlation was observed between our classification results and the behavioral data (regression analysis: $R^2 = 0.6982$; $P = 0.0026$; $n = 10$ data points). Hence, this result reveals a direct relationship between dynamic processing of odor signals in the antennal lobe and recognition performance of locusts in a behavioral task.
We found that although citral was not suitable as a conditioned odor in the appetitive-conditioning assay, this odor repelled locusts in a T-maze assay (see Methods in Chapter 2). Fortuitously, the corresponding background odor used in our physiology experiments (geraniol) elicited an exact opposite innate response and functioned as an attractant in the T-maze assay. Both dimensionality reduction analysis and classification results had revealed that the geraniol responses masked any subsequent citral introductions when these two odors were presented in an overlapping fashion. This result suggests that a mixture of these two odors should attract locusts in the T-maze assay (Fig. 3.9a). We tested this prediction by presenting citral two seconds following geraniol introductions and found that locusts were indeed attracted towards the T-maze arm that delivered this stimulus (Fig. 3.9b; \( P < 0.05 \) exact binomial test with Bonferroni correction for multiple comparisons; \( n = 20 \) locusts for each test stimulus). Hence, integrating our physiology results with behavioral data, we conclude that the ensemble PN firing patterns underlie both acquired as well as innate preferences in this olfactory system.
Figure 3.9 Behavioral results of T-maze assays vs. physiology data. (a) Result bar plots from a T-maze assay showing preferences of locust to the three test stimuli delivered: citral or geraniol or geraniol – 2 s lag – citral. The response to geraniol and the geraniol–citral sequence were both significantly different from the citral response (exact binomial test; * $P < 0.05$ with Bonferroni correction for multiple comparisons; $n = 20$ locusts for each case). (b) PN classification probabilities for the same set of stimuli used in the T-maze experiments are shown (mean classification probability computed from curves shown in Fig. 3.6e right panel).

3.2.7 Network Property Determines Odor Recognition

Thus far, our data revealed that in 5-out-of-6 odor pairs tested, the foreground odors can be tracked independent of the presence of the background odor. However, for the citral-geraniol odor pair, none of the citral introductions atop geraniol were effectively tracked (Fig. 3.4e, 3.5e, 3.6e). We identified that amongst all foreground odors tested so far, citral evoked less excitatory and more inhibitory responses in most PNs. Furthermore, citral also had a weaker PSTH compared to the geraniol response. Therefore, when presented atop an on-going background odor, a strong input appeared necessary to overcome the resistance offered by the on-going neural activity and switch the population response from the background attractor to a different subspace that represented the foreground odor.
Furthermore, we hypothesized that two independent factors that may determine whether the foreground odor could be recognized in a background-invariant manner: 1) the intrinsic property: net excitatory vs. inhibitory activity evoked by the foreground odor; 2) the network property: overall foreground vs. background PN activity. To examine which of this hypothesis determines background-invariant recognition, we selected two representative odor pairs (iaa-bzald, cit-ger) and varied the concentrations (0.001%, 0.01%, 0.1%, and 1% v/v concentrations) of the either the background odor (ger) or the foreground (iaa) while keeping the other odor in the sequence a constant. Again, the same qualitative dimensionality reduction analysis and quantitative classification analysis were employed to validate our hypothesis (Fig. 3.10). Our results reveal that as the iaa concentrations were reduced below a threshold level (0.1% dilutions), iaa introductions atop bzald background did not reorganize the ensemble neural activity to follow the freshly introduced stimulus. On the other hand, when ger intensities were reduced below (< 1% dilutions) a threshold level, introduction of cit at 1% effective reorganized to follow the newer odorant. These results suggest that the ability to track a novel odor atop a background stimulus is a result of dynamic interaction between the two competing cues. Further, as long as the freshly introduced stimulus generates stronger overall response than the background odor, the antennal lobe network can switch to track the odorants in a background-invariant manner. Further, background-invariant encoding for an odor is determined completely by the antennal lobe network property, since lowering background odor intensity (Fig. 3.10a) or increasing the foreground odor intensity (Fig. 3.10b) switched the competition in favor of the foreground odor.
Figure 3.10 Background-foreground odor pairs with varying concentrations. (a) Left panel: Same odor trajectory analysis as in Fig. 3.4 was performed on the cit-ger odor pair with gradient background concentrations of 0.001%, 0.01%, 0.1%, and 1%. For each combination, the foreground concentration of cit was fixed at 1%. Middle panel: Same classification analysis as in Fig. 3.6 was conducted for each odor combination that corresponded to the conditions shown in left panel. Right panel: classification probabilities for each time bin; same analysis as in Fig. 3.6 right panel. (b) For iaa-bzald odor combination with the foreground iaa concentrations of 0.001%, 0.01%, 0.1%, and 1% and background bzald at 1% concentration.
3.3 Discussion

We examined how a spatiotemporal coding mechanism allows an olfactory system to detect and recognize olfactory cues in the presence of another competing stimulus. Our results revealed that ORNs’ responses to an odorant changed when the same stimulus was presented along with or following another stimulus. Subsequent processing of these inconsistent sensory inputs varied depending on the latency with which the fresher stimuli were introduced. However, we found that following most foreground odor introductions the antennal lobe ensemble activity restructured to create neural activity that overlapped across different presentation conditions. Piecewise decoding of these responses by KCs allowed robust detection of any similarity in the antennal lobe ensemble activity.

Our study also examined when the olfactory system achieved the ability to process a newer stimulus independent of its recent stimulus history. This is important because in most natural settings the latency with which a fresh odorant is received cannot be controlled. Therefore, we presented the newer stimulus during all possible dynamic states of neural activity elicited by a preceding odor. Note that for cases when the two odor onsets happened within a few hundred milliseconds of each other, neither the sensory input from ORNs[67, 126, 127] nor the behavioral response would have attained complete adaptation (although behavioral responses can change extremely rapidly[128, 129]). Nevertheless, our results revealed that when following another odorant, either all introductions of fresher stimuli were tracked independent of the latency with which they were introduced, or none of them were tracked.

We found that rapid filtering of background odor signals began at the level of ORNs. A lengthy odor pulse did not elicit a similarly lengthy response in all ORNs. A subset of ORNs showed a transient response that substantially reduced within a second after odor onset, while
another subset showed a response that persisted for the complete duration of the odor pulse (Fig. 3.2a). Since the same odor could elicit both transient and persistent responses in different subsets of ORNs, these temporal response properties cannot be explained by variations in stimulus dynamics alone[130]. These results suggest that rapid adaptation at the level of sensory neurons may contribute to filtering of background stimuli and facilitate detection of novel odor introductions.

We found that even though spiking activity in individual ORNs was greater, when overlapping pairs of stimuli were presented, individual PN responses to these blends were lower than the responses to the foreground stimulus presented alone (Fig. 3.3b). Surprisingly, during off-transient introductions of foreground odors, a relatively weaker ORN input was able to elicit a comparatively stronger PN firing activity. Our behavior results also revealed that in some cases the off-transient activity due to a background stimulus could interfere with subsequent odor recognition (Fig. 3.8c; hex–2oct; $P = 2.289 \times 10^{-5}$, McNemar’s exact tests with Bonferroni correction for multiple comparisons, $n = 50$ locusts). These results suggest that the sensory neuron activity alone is insufficient to completely understand the responses generated in the following circuits.

We found that responses of a large subset of PNs to the foreground odor remained unaffected due to changes in stimulus history (~36% of responsive cells; data not shown). Such a PN subset existed even for the ger–cit odor pair where none of the citral introductions were effectively tracked by the ensemble PN activity. Hence, we conclude that the existence of this subset, though necessary, by itself was insufficient to ensure that the ensemble activity overlapped across conditions.
Most of our PN analyses discussed here rely on pooling data across multiple locusts. Does this analysis approach impact our conclusions? Several lines of evidence point to the fact that pooling data would lead to more meaningful interpretation of the dataset as it does not suffer from subsampling effects. Empirically, in agreement with previous studies[69, 75], we found that our classification results converged when using roughly 80 PNs (data not shown). Furthermore, Monte-Carlo simulations revealed that the probability of double-counting a stereotypic PN across locusts (if they existed) was extremely low. Therefore, we expect this pooling strategy to be well-founded for the objectives of this study.

We found that, for all odor-pairs, a subset of KCs responded to any overlap observed in the antennal lobe ensemble activity. Even for those foreground odors where the overlap across conditions occurred in non-identical response segments (e.g. 2oct–hex in Fig. 3.4), we found KCs that responded in an invariant manner (Fig. 3.7; KC1, KC2). We also found a few KCs that responded to only a subset of overlapping conditions (Fig. 3.7; ger–cit). Such KC responses were consistent with the classification analysis results for that odor pair. Hence, our data supports the existing interpretation of KCs as a piecewise decoder of ensemble PN activity[75, 76]

Our physiology results alone were not sufficient to identify the attributes of the spatiotemporal ensemble activity that allow background independent recognition of odors. Given these results, several possible behavioral outcomes can be anticipated. For example, since an exact match between the entire set of spatiotemporal responses was not achieved in any of the CS presentation conditions, one possible outcome is that locusts will not recognize subsequent introductions of trained odor following a distractant. Alternately, whether or not the neural activity returned to baseline levels prior to the onset of a fresher stimulus (i.e. temporal decoupling) may be important for robust recognition. If the latter case was indeed true then we
would expect a difference in recognition performance between the on-transient and steady-state introductions of the same CS. Notably, our behavior results appear to indicate a rather simple approach. Any partial pattern-match with or without a reset in the neural activity was sufficient for recognition of CS by trained locusts (Fig. 3.8).

In conclusion, our results reveal that an odorant evoked only certain combinations of ensemble neural activity in the antennal lobe that encoded for its identity (a ‘subspace’ or an ‘attractor’). The neural response dynamics during on-transients and steady-state periods were both contained within this subspace for a given pure odor stimulation. When presented atop an on-going background odor, a strong excitatory input appeared necessary to overcome the resistance offered by the on-going neural activity and switch the population response from the background attractor to a different subspace that represented the foreground odor. This interpretation is well supported by the analysis results of the dataset with varying odor concentrations (Fig. 3.10). Notably, we found that reaching any segment of the attractor was sufficient to allow robust recognition. Hence, our results provide a fundamental insight on a behaviorally important olfactory computation.

3.4 Author Contributions


B.R. conceived the study and designed the experiments. D.S., K.L. and G.S. performed the electrophysiological recordings. C.L. and S.P. did the behavioral experiments. C.L., K.L., and D.S. analyzed the data. B.R. wrote the paper and D.S., C.L. and K.L. provided feedback on the manuscript.
Chapter 4: Behavioral Correlates of Combinatorial versus Temporal Features of Odor Codes

4.1 Introduction

Time and space are fundamental neural coding dimensions. Sensory cues, even stationary ones, often activate an ensemble of neurons with a precise temporal structure. Determining what features of a stimulus are encoded by the active set of neurons (‘combinatorial code’) and what aspects are represented in their temporal structure (‘temporal code’) is a fundamental problem in systems neuroscience. Alternatively, these two dimensions may not independently encode information. In this case, the joint spatiotemporal patterns of spiking activity could provide a large coding space for representing stimuli [25, 26]. Identifying the right coding scheme employed by a sensory system is essential for determining the rules that govern how stimulus-evoked neural activity is translated to a behavioral response.

In the insect olfactory system, olfactory sensory neurons in the antenna transduce chemical cues into electrical signals and transmit them to the antennal lobe neural circuits (analogous to the olfactory bulb in vertebrates) for further processing[25, 26, 69]. Previous studies have shown that odorants activate temporally structured principal neuron responses in the antennal lobe (and in the olfactory bulb) that vary with and therefore have the capacity to encode for stimulus identity and intensity [62, 68, 69]. However, these response patterns are disrupted by hysteresis arising from stimulus dynamics[75, 124] and recent history[76]. Furthermore, to date there is no behavioral evidence to suggest that insects use the temporal structure of neural responses for odor recognition.
On the other hand, a purely combinatorial code offers greater encoding capacity than a labeled-line scheme where a set of neurons exclusively represents a stimulus. However, adapting the sensory system to ignore a redundant stimulus becomes difficult, as suppressing responses to one odorant will also alter the neural activity evoked by a number of other stimuli. How then can the system preserve the identity of a cross-adapted odorant?

Here we sought to investigate these issues using the locust olfactory system. We show that although combinatorial and temporal codes by themselves have potential deficiencies, together they can allow robust encoding of stimulus identity and facilitate emphasizing or deemphasizing certain stimuli based on their novelty or lack thereof. Furthermore, we reveal how information contained in the combinatorial and temporal features of stimulus-evoked activities gets translated to behavior.

### 4.2 Results

#### 4.2.1 Stimulus History can Disrupt Temporal Response Patterns

We began by analyzing the responses of projection neurons in the antennal lobe to lengthy but solitary presentations of different odorants. Both simple (monomolecular) and complex cues were used. In general, we found that the onset of stimulus-evoked activity was rapid with 60% – 90% of responsive neurons reaching above baseline levels within 600 ms of odorant onset (see Methods of chapter 2 for response latency). The median response latency was in 300 – 400 ms range for all odorants used except citral that elicited a comparatively slower response (550 ms median latency).

We found that lengthy odor exposures generated temporally patterned spike trains in individual projection neurons (Fig. 4.1a, c), and that different neurons had different patterns of
stimulus-evoked responses. For example the spike rasters for PN4-PN6 for Locust 2 (Fig. 4.1a, middle column) exhibit markedly different activity patterns in response to the same odorant (hexanol). However, as an ensemble, the projection neurons tended to reach their peak response values in a coherent fashion. This was true whether the neurons were recorded simultaneously from a single locust (Fig. 4.1a), or pooled across multiple animals (Fig. 4.1c). As a consequence, during this period of high stimulus-evoked activity, coincident spikes across projection neurons were observed for a wide variety of chemical cues tested. Qualitatively, the number of neurons that contributed to synchronous firings and the time periods when such activities were observed for each odorant can be identified by vertical alignment of tick marks in the raster plots showing spiking activity across neurons (Fig. 4.1c).

In natural conditions, odorants are seldom encountered in isolation and are typically preceded and succeeded by other chemosensory cues. Could the observed neural response coherence be disrupted when odorants are not encountered in isolation but presented following another stimulus with some overlap? To examine this, we delivered the same set of stimuli in an overlapping sequence preceded by another pulse of the same or a different odorant. We delayed introduction of the second pulse until the response to the first pulse had reached a low, but persistent state (or ‘steady-state’ activity[66, 67, 125]). Note that the first stimulus was not terminated prior to or during the second stimulus exposure. This was done to prevent offset responses due to termination of the first stimulus from combining with responses evoked by the succeeding stimulus in the sequence. This stimulation protocol allowed us to compare the ensemble spiking responses following solitary introduction of a stimulus with those elicited by the same stimulus when delivered in an overlapping sequence.
Figure 4.1 Odor-elicited neural synchrony in the antennal lobe. (a) Projection neuron (PN) spiking responses evoked by solitary odor introductions are shown for ten consecutive trials. Each row reveals spiking patterns during 4 s odor presentation with a 500 ms pre-stimulus period. The mean spike counts as a function of time (PSTH; 50 ms time bins) are shown in the right panel. Note that the three PNs shown in a column were simultaneously recorded from a single locust. (b) The same set of PNs to the same set of odorants as in panel a are shown but the odorants are presented in an overlapping sequence. The 4 s period of stimulus overlaps are shaded in gray, whereas a blue shade is used to represent a 500 ms time period when the first stimulus in the sequence is present alone. (c) Spiking activities of PNs (pooled across experiments; 10 trials each) with significant responses to solitary odor presentation are shown. PNs were sorted based on their average latency to peak response. Arrows along x-axis indicate the time of odor onset. Two different PN ensemble responses shown for iaa and three groups of hexanol were probed with different odorant combinations. (d) Responses of same set of PNs as in panel c but in an overlapping sequence. (e) Red traces represent spike counts across all PNs following solitary odor introduction, and...
the black traces show the spike counts across the same set of PNs by the same odorant but presented in an overlapping sequence (mean ± s.d.; \( n = 10 \) trials, bin size = 10 ms). Asterisks indicate significant reductions in peak spike counts (*\( P < 0.01 \), paired t-tests, \( n = 10 \) trials). (f) Spike counts summed over the entire duration of odor presentation (4 s) are shown for all PNs during solitary (in red) and overlapping (in black) stimulus conditions (mean ± s.d.; \( n = 10 \) trials). Correlation coefficients (R) were calculated between PN response profiles shown in black and red. Insets show the total spike-count across all PNs during solitary and overlapping presentation of the same odor (mean ± s.d.). Asterisks indicate significant change in total spike counts (*\( P < 0.01 \), NS: \( P > 0.01 \), paired t-tests, \( n = 10 \) trials). (g) Correlation between spike-count profiles across PNs (see Methods of chapter 2) are shown for: (i) inter-trial responses (striped bars), (ii) inter-condition responses (solitary vs. overlapping introductions; solid bars), and (iii) inter-odor responses (open bars). (Two-way ANOVA, \( P(\text{inter-trial vs. inter-condition}) = 0.12 \) (Bonferroni corrected for multiple comparisons), \( P(\text{inter-trial vs. inter-odor}) = 1.53 \times 10^{-5} \), \( P(\text{inter-conditions vs. inter-odor}) = 7.25 \times 10^{-4} \)).

We found that temporal structure of the spike trains generated by an odorant could change depending on whether the stimulus was presented in isolation or introduced following another odorant (Fig. 4.1a vs. Fig. 4.1b; note, we refer to the activity following the introduction of the second pulse as second-odorant((first-odorant)). Interestingly, for some odor sequences (e.g. iaa vs. iaa(bzald)), the temporal response patterns were robustly maintained.

At the neural ensemble level, as expected, the spiking responses to the onset of the first odorant were synchronized. However, in most cases, the temporal structures of neural activities following the introduction of the second stimulus in the sequence were less coherent across neurons compared to the responses elicited during solitary introductions (Fig. 4.1b, d). We found that different neurons reached their peaks at different time points (Fig. 4.2). Hence, the second odorant in the sequence was less likely to elicit coincident spikes across projection neurons (Fig. 4.1b, d).
Figure 4.2 Altering stimulus history can disrupt temporal coherence of ensemble neural activity. (a) PN ensemble responses to solitary and overlapping presentations of six odors are shown. Peristimulus time histograms (PSTHs) were averaged over trials and are shown as gray scale images for responsive projection neurons (see Methods of Chapter). Each row reveals the response of a single projection neuron during the four seconds of odor exposure (80 time bins). Red triangles mark the peak of each projection neuron PSTH. Vertical alignment of the red triangle markers indicates that a large fraction of projection neurons reach their peak response in a highly coherent manner for all solitary odors introductions. For comparison, the bottom panels show ensemble responses to the same set of odorants but presented in an overlapping sequence with another odorant (projection neuron ordering is same in both panels). Note that the peak of the PSTH response to the second stimulus in the sequence happens with different latencies across the ensemble. The fraction of PNs reaching their peak value in a given 250 ms time bin is shown for different odorants (time-to-peak-response distribution). The time-to-peak-response distribution for solitary introductions is shown in red and the response distribution for overlapping odor introductions is shown in black. Significant differences in time-to-peak-response distribution variances were determined using Levene’s test. (b) Similar plots as in panel a, but showing responses of three odorants that generated synchronous activity when delivered solitarily or in an overlapping fashion.

To quantify these results, we counted the number of spikes observed across the neural ensemble in 10 ms non-overlapping time bins (red traces in Fig. 4.1e). Note that coincident spikes across neurons result in the larger spike counts in a given time bin and therefore can be used as a measure of neural response synchrony. These results confirm that solitary presentations of any odorant generated synchronous spikes across an ensemble of projection neurons in the antennal lobe. Compared to responses evoked during solitary presentations, the degree of spike synchrony for most odorants was significantly reduced when they were introduced following another stimulus (black traces in Fig. 4.1e; paired t-test, * indicates $P < 0.01$, $n = 10$ trials). Surprisingly, for three out of the nine odor sequences presented in this fashion, the ensemble response to the second pulse was as synchronous as the responses evoked by their solitary presentations (refer to iaa(bzald), hex(hxa) and 2hep(chex) cases in Fig. 4.1). Taken together, these results indicate that the same set of stimuli can generate neural activities with different temporal structures in the antennal lobe depending on whether they were introduced solitarily or in an overlapping sequence.
4.2.2 Combinatorial Odor Code Robustly Encodes Odor Identity

Next, we examined whether the activated set of neurons was altered between solitary versus overlapping presentations of the same odorant. We computed the spike counts of each individual projection neuron during the entire odor duration, i.e. following solitary introduction of the odorant or following the introduction of the same odorant as the second stimulus in the sequence. We then compared the ensemble activation profiles across the two presentation conditions (Fig. 4.1f; red (solitary) vs. black (overlap) curves). Although there were some variations, the overall projection neuron ensemble activation profile during solitary and overlapping introductions was surprisingly well conserved for most odorants (Fig. 4.1f). Furthermore, the correlation between ensemble activation profiles across different presentation conditions was comparable to those observed across different trials of the same stimulus (Fig. 4.1g; two-way ANOVA with Bonferroni correction for multiple comparisons, $P = 0.12$). Both correlation across different trials and across different conditions were significantly higher than those observed between response profiles generated by different odorants (Fig. 4.1g; two-way ANOVA, $P(\text{inter-trial vs. inter-odor}) = 1.53 \times 10^{-5}$, $P(\text{inter-conditions vs. inter-odor}) = 7.25 \times 10^{-4}$).

Hence, our results indicate that with respect to variations in how a stimulus is encountered the combinatorial features of ensemble activity are more robust than temporal response patterns.

4.2.3 Response Magnitude versus Neural Synchrony

It is possible that the reduction in spike synchrony across neurons may simply arise due to diminished spike counts observed during sequential introduction of some odorants. To test this hypothesis, we compared the spike counts elicited by an odorant during solitary versus
overlapping presentation conditions. At the individual projection neuron level, we found that the total spike counts could significantly increase/decrease or remain consistent across presentation conditions (Fig. 4.3a; paired t-test, at 0.05 confidence level). The distributions of number of projection neurons with increase/decrease/no change across presentation conditions were comparable across different odor pairs used in the study (Fig. 4.3b; two-way ANOVA, $P = 1.0$).
Figure 4.3 Spike count analysis of individual projection neurons. (a) Comparison of mean projection neuron spike counts in a 4 s window after solitary introduction of an odorant or following the introduction of the same odorant as the second stimulus in an overlapping sequence. The x axis corresponds to spike counts when the odor is presented alone. The y axis corresponds to spike counts when the odor is presented after a preceding stimulus. The mean ± s.e.m. over ten trials is shown for all cells. Cells in orange indicate a significant increase in spike counts during the overlapping conditions and are therefore located above the diagonal (P < 0.05, paired t-test). Similarly, cells in purple indicate a significant decrease in spike counts, and cells in gray indicate no significant change across the two conditions. (b) Bar plot showing the number of cells with significant increase, decrease or no change across presentation conditions for the different odorants used in the study. No significant difference was observed between distributions computed for different odor groups (two-way ANOVA; P = 1.0).

At the ensemble level, we again found that the total spike counts across neurons can also significantly increase/decrease or remain consistent across presentation conditions (Fig. 4.1f insets; paired t-tests, * indicates P < 0.01). Notably, even when the overall spike synchrony did not diminish (e.g. iaa vs. iaa(bzald)), we found that the total spike counts across neurons can decrease significantly during overlapping presentations of the stimulus (Fig. 4.4b). Alternately, we found cases where the overall spike synchrony reduced significantly (e.g. 2oct vs. 2oct(hex)), but the total spike counts across neurons remained consistent across conditions (Fig. 4.4a,b).

We found that there was no significant correlation between reduction in spike synchrony and reduction in spike counts (Fig. 4.4a-c; linear regression analysis, $R^2 = 0.11$, $P = 0.37$). Hence, we conclude that the observed reduction in the spike synchrony across neurons (Fig. 4.1e) cannot be explained based on changes in the response magnitude during solitary versus overlapping introductions of the same stimulus.

4.2.4 Cross-talk Between Stimulus-evoked Responses

Are there rules that govern when the response to a preceding stimulus diminishes the coherence in ensemble activity evoked by a succeeding stimulus? We hypothesized that this cross-talk between neural activities evoked by the two stimuli could be a result of their response
overlap. Therefore, we first computed the percentage of projection neurons that were activated by both odorants presented in a sequence (see Methods of Chapter 2). Since odorants evoked a combinatorial response in this network, in general, we found that any two odorants had some amount of overlap. However, for those odor pairs where disruption in synchrony was observed, we found that the ensemble response overlap was considerably greater (Fig. 4.4a, d). A significant linear relationship was observed between projection neuron response overlap and reduction in neural synchrony (Fig. 4.4e; linear regression analysis, $R^2 = 0.82, P = 7 \times 10^{-4}$).

Figure 4.4 Rules for the changes in neural synchrony. (a) Percentage reduction in the peak spike synchrony between solitary vs. overlapping introductions of the same odorant is shown (mean ± s.e.m.; $n = 10$ trials). The bars are shaded based on the significance of peak neural synchrony reduction (logarithms of their $P$ values). (b) Percentage reduction in total spikes across neurons is shown for solitary vs. overlapping introduction of the same odorant (area under black curve/area under red curve shown in Fig. 4.1f). Same color convention as in panel a. (c) Regression analysis between spike-count reduction (x-axis; panel b) and reduction in peak synchrony (y-axis; panel a) did not reveal any significant relationship between variables ($R^2 = 0.11, P = 0.37$). (d) Percentage of neurons activated by solitary presentations of both odorants is shown (see Methods of Chapter 2). Same color convention as in panel a. (e) Regression analysis between response overlap as quantified by the percentage of co-activation (x-axis; panel d) and reduction in neural response synchrony (y-axis; panel a) revealed a significant linear relationship between the two variables ($R^2 = 0.82, P = 7 \times 10^{-4}$).
Notably, this cross-talk was not determined by chemical similarity (same vs. different functional groups), behavioral similarity (geraniol is an attractant and citral is a repellant), or odor delivery sequence (hex(2oct) vs. 2oct(hex)), but was purely determined by the neural response overlaps (an inverse measure of the novelty of the stimulus). Hence, these results reveal that when encountering a series of stimuli, only those odorants that were less redundant, or more novel, evoked synchronous activity in the antennal lobe.

4.2.5 Odor Identity can be Robustly Maintained across Conditions

Does synchronization or desynchronization of ensemble neural responses alter the overall representation and therefore the encoded identity of the odorant? To understand this, we performed a qualitative dimensionality reduction analysis[69] (Fig. 4.5a; see Methods of Chapter 2). We constructed a time-series of high dimensional projection neuron ensemble response vectors, where individual vector elements corresponded to spike counts of a single neuron in a given 50 ms time bin. These high-dimensional vectors were projected onto three dimensions using a linear principal component analysis technique. The low dimensional points were connected in a temporal order to visualize the neural response trajectories.

We found that each odor evoked a closed loop trajectory in a certain direction. When presented following another stimulus, the responses following the introduction of the second stimulus in the sequence (black trajectories) were still aligned with the firing patterns elicited by solitary presentations of the same odor (red trajectories). However, for those odorants that had greater response overlaps with the preceding stimulus, the intensity of the response (i.e. the length of the vectors) was substantially reduced. As expected, the length of the response vectors depended on whether the responses were synchronous or asynchronous across neurons. On the other hand, the response pattern match between solitary and overlapping presentations depended
predominantly on whether the set of neurons activated (i.e. the combinatorial code) remained consistent across presentation conditions (Fig. 4.1g). We found that when the combinatorial code was altered significantly (for example, cit vs. cit(ger) case; Fig. 4.5a), the pattern match also diminished across conditions. The qualitative dimensionality reduction results were quantified using a classification analysis (Fig. 4.5b; pattern matching done purely using vector similarity; see Methods of Chapter 2). In sum, these results confirm that odor identity is robustly represented when only the temporal structure varies between ensemble responses elicited during isolated (synchronous response; Fig. 4.1a, c) and overlapping presentations (asynchronous response; Fig. 4.1b, d) of the same stimulus.

4.2.6 Decoding Different Modes of Antennal Lobe Responses

To understand how disruption in ensemble neural synchrony alters responses in the neural population downstream to the antennal lobe circuits, we monitored Kenyon cell responses in the insect mushroom body. We found that Kenyon cells were more likely to respond to an odorant during the periods of peak antennal lobe activity. Furthermore, they responded to an odorant irrespective of whether the odorant was delivered in isolation or succeeding another odorant (Fig. 4.6a1, b1; paired t-test, * indicates $P < 0.01$). However, the probability of response was significantly reduced for those odorants that failed to elicit a highly coherent antennal lobe response (Fig. 4.6a2,a3 vs. b2,b3). Hence, these results are consistent with the idea that synchronous neural inputs are likely to more reliably activate target cells[131-133].
Figure 4.5 Effects of desynchronization on response intensity and identity. (a) Evolution of odor evoked ensemble responses over time are shown after dimensionality reduction using principal component analysis. The number of neurons (n) used for this analysis and the percentage of variance captured in the first three dimensions are shown in each plot. Numbers near response trajectories indicate time in seconds since odor onset. Red and blue trajectories are solitary introductions of two different odorants. Black trajectories reveal the ensemble responses following the introduction of the second odorant in the sequence. (b) Blue and red traces indicate pattern match between ensemble activities generated following the introduction of the second odorant with the response templates obtained for the component odorants (see Methods). Note that the stimulus identity is robustly maintained for all overlapping cases except cit(ger).
Figure 4.6 KC spiking probabilities to different stimulus conditions. (a1) Representative raster plots revealing responses of two different Kenyon cells in the insect mushroom body to odorants that do not produce coherent PN response during odor overlaps. (a2) Kenyon cell PSTHs (mean ± s.d.; n = 10 trials) are shown as a function of time for solitary (red) and overlapping (black) introductions of six different odorants (70 Kenyon cells in total; *p < 0.01, paired t-test for peak firing rate comparison). Note that the six odorants used are those that evoked antennal lobe ensemble responses that were less synchronous when presented following another stimulus. (a3) Left, response of each Kenyon cell (KC) is shown for single and overlapping presentations of different odorants. Black represents ‘responsive’ and white represents ‘non-responsive’ KCs (see Methods in Chapter 2). Right, fraction of KCs that responded to different introductions of the same set of odorants is summarized. All KCs recorded for odor pairs that elicited asynchronous PN ensemble activity in the antennal lobe were combined for this analysis. (b1, b2, b3) Similar plots as panels c1, c2, c3 but analyzing all KC responses to those odorants that revealed coherent projection neuron responses no matter how they were introduced.

4.2.7 Behavioral Correlates of Neural Synchrony

Finally, we examined whether the observed synchrony in ensemble neural response was relevant to odor-evoked behavior in this model system. To investigate this, we trained locusts in an appetitive-conditioning assay[92]. During the training phase, an odorant (conditioned stimulus; hexanol or isoamyl acetate) was presented with a grass reward (unconditioned stimulus). Selective opening of the maxillary palps (sensory appendages close to the locust mouth) to the conditioned stimulus presentations in the unrewarded test trials served as an indicator of acquired memory (see Methods of Chapter 2).

To perform fine-grained analysis of the behavioral response, we painted both the palps and tracked their movement with 100 ms temporal resolution using a custom-written image processing software. We found that trained locusts responded to the conditioned odor reliably and for the entire duration of the odor pulse (Fig. 4.7a; 1st column). The response onset was rapid and consistent across locusts (Median time = 0.70±0.27 s for hex and 0.60±0.39 s for iaa; see Methods of Chapter 2). We found that locusts trained with hex also responded to introductions of 2-octanol, an odorant that evoked a highly overlapping neural response (Fig. 4.7a; 2nd row). Note, however, that the trained odor (hex) elicited a stronger response than the untrained odor (2-octanol). Conversely, locusts trained with isoamyl acetate, selectively responded to the trained
We took advantage of our earlier results that revealed that locusts performed consistently when multiple unrewarded test trials were carried out in a back-to-back fashion (see Fig. 3.8b). Matching our electrophysiology studies, we delivered pulses of a trained stimulus in a solitary or an overlapping fashion. Pulses of the same or differing odorants (hex(hex), hex(2oct), or iaa(bzald)) with two different delays (2 s and 4 s) were used to evaluate behavioral performance (Fig. 4.7a second and third columns). The four seconds delay was used to further facilitate decoupling of the behavioral responses elicited by the two stimuli delivered in quick succession.

We found that introductions of the trained stimulus (in isolation or in an overlapping sequence) elicited a distinct and significant palp-opening response in all cases (Fig. 4.7a; Wilcoxon signed-rank test, * indicates P < 0.05). Considering that these stimulus introductions elicited ensemble responses with completely different temporal structure (Fig. 4.1a-d), this result is consistent with our interpretation from physiology data that stimulus identity is robust to variations in the temporal structure of the ensemble activities.
Figure 4.7 Behavioral response predictability vs. neural synchrony. (a) PORs tracked during test phase trials are shown for each type of stimulus presented (mean ± s.e.m). The conditioned stimuli (hex or iaa) were either presented alone (left column) or following another odorant with two different latencies: 2 seconds (middle column) or 4 seconds (right column). Responses to solitary presentations of the two untrained odorants (2oct and bzald) are plotted in blue in the leftmost column. Asterisks indicate
significant increases in the peak POR elicited by the second stimulus (*$P<0.05$, Wilcoxon signed-rank test, $n$ indicates the number of insects used in the test). (b) Pairwise correlation between PORs (a measure of behavior predictability) observed in different locusts were calculated as a function of time (mean ± s.e.m.). The red traces quantify neural synchrony for the same set of odor stimuli (spike counts in 100 ms time bins were used to match the behavioral response resolution). Following conditioned stimulus introductions, significant correlations were observed between physiology and behavioral data for all conditions tested (Pearson’s correlation coefficient; $n = 60$ time bins including 4 s of odor exposures and 2 s of offset responses). (c) Maximum value of the pairwise correlation to solitary and overlapping presentations of different conditioned stimuli are summarized (mean ± s.e.m.). Asterisks indicate significant change in pairwise correlation between PORs (*$P<0.05$, NS is $P > 0.05$; Wilcoxon signed-rank test with Bonferroni correction for multiple comparisons; $n = 171, 465, 210$ pairs of locusts). (d) Comparison of the peak neural synchrony are shown for those stimuli also used in the POR assay (mean ± s.e.m.; $n = 10$ trials). Asterisks indicate significant reductions (*$P<0.05$, NS is $P > 0.05$, paired t-test).

To understand how predictable the locust palp-opening responses were following introduction of the trained odorant, we computed the pairwise correlation between the behavioral responses observed in different locusts in a specific time segment (Fig. 4.7b; see Methods of Chapter 2). The larger the pairwise correlation in a given time segment, the greater the predictability of the response based on responses observed in other locusts. Remarkably, a significant correlation was observed between the predictability of the behavioral responses to the conditioned stimulus and the neural response synchrony (as quantified by the summed spiking activity across neurons; Fig. 4.7b black vs. red traces and Fig. 4.7c,d). Note that the behavioral responses of locusts were highly predictable at odor onsets and offsets for both conditioned stimuli. During middle portions of an odor stimulus or when responding to the second pulse of hexanol, the neural synchrony was diminished and the behavioral responses, although significantly above the baseline levels, were less predictable across locusts. Increasing the delay with which the second pulse of hexanol was introduced did not improve behavioral response predictability (Fig. 4.7c). Notably, for isoamyl acetate, the neural synchrony was not disrupted when presented in sequence with benzaldehyde. Correspondingly, the behavioral response was highly predictable for all isoamyl acetate introductions. Therefore, we conclude that the
behavioral consequence of disrupting neural synchrony is not in eliminating the palp-opening responses altogether but in making them highly variable. In sum, our results reveal a direct correlation between changes in neural synchrony and behavioral response predictability in the locust olfactory system.

4.2.8 Combinatorial versus Temporal Odor Code

Our physiology results revealed that the temporal structure of a stimulus could be disrupted by stimulus history. However, the odor identity was robustly represented by the ensemble neural responses. How is this achieved? To develop an intuition behind this neural computation, we developed a simple ensemble neural model. We modeled the response of each neuron to a stimulus using an exponential function with just two parameters: response amplitude and response time constant. Different stimuli were modeled to activate partially overlapping subsets of neurons (see Methods of Chapter 2).

We found that the disruption of temporal structure diminished the overall response intensity as shown by the response trajectories that dwarfed after the introduction of experimentally constrained variable delays (Fig. 4.8). However both jittered and non-jittered versions of ensemble neural activities were still aligned and occupied the same subspace as shown by the dimensionality reduction analysis. On the other hand, a substantial change in the activated group of neurons resulted in a mismatch of population-level responses (refer to cit-ger trajectories for example; Fig. 4.1f, 4.5). Hence, our results support the idea that as long as a conserved set of neurons is activated the stimulus identity can be insensitive to variations in the temporal structure of their neural firing. This result also suggests that spatial (identity) and temporal (novelty, intensity) features of neural responses may be used for encoding non-redundant aspects of a stimulus.
Figure 4.8 Combinatorial vs. temporal properties of neural activity. (a) Simulated neural activities in a well-constrained computational model with 100 neurons are shown for a single odorant that activated the same set of neurons but with different temporal structures (left vs. right panel). The fraction of neurons reaching their peak values at any given time period is shown below each image plot. (b) Similar plots as in panel a but now showing responses to a different simulated odorant. Note that the two odorants shown in panels a, b activate different but partially overlapping subsets of neurons. (c) The more synchronous the ensemble activity, the greater was the overall response as measured by the mean PSTH (± s.e.m.). Asterisks indicate significant difference in spike counts across neurons (* P < 0.05, Wilcoxon signed-rank test, n = 100). (d) A linear dimensionality reduction analysis of the simulated neural data revealed that odorants that activated the same set of neurons traced response trajectories that were aligned with each other irrespective of their differences in temporal structure.

4.3 Discussion
In neural circuits, coordinated spiking activities across ensembles of neurons have been observed at a variety of temporal and spatial resolutions [50, 134-141]. Such temporally precise responses have been shown to be important for learning[142-144] and are thought to mediate various rhythmic field-potential activities observed at different oscillation frequencies in the brain[145]. However, whether synchronous neural activities facilitate different neural
computations compared to asynchronous responses and whether different modes of neural transmission produce different types of behavioral responses remain unclear.

One prevalent hypothesis is that synchronous neural signals may provide a substrate to integrate pieces of information individually encoded by neurons (‘the neural binding’ problem[146]), and are therefore considered important for neural computations[146-148]. This hypothesis, however, is still widely debated[149-151]. Furthermore, recent works have shown that stimulus-evoked neural activity can also be asynchronous in nature[152-155] where temporal integration rather than coincidence detection has been proposed as a readout mechanism[152]. These observations further confound the functional role of coordinated spiking activity across neurons. Can a stimulus evoke both synchronous and asynchronous ensemble responses, and if so, when is a particular mode of neural activity preferred to transmit information? Our results describe an interesting mechanism based on synchrony that allows neural networks employing a combinatorial coding scheme to selectively deemphasize certain sensory inputs. Notably, as shown in our computational model, desynchronizing ensemble activities only reduced the intensity of the circuit’s response to the adapting and cross-adapting stimuli without altering their encoded identity.

We examined the relationship between spike synchrony we observed with other forms of neural synchrony reported in these circuits. Previous studies have shown that odorants also entrain oscillatory synchronization of projection neuron activities in the insect antennal lobe[26, 49-52]. This neural synchronization resulted in field potential activity with power in the gamma range (~20 Hz) and has been shown to slowly build up with repeated presentation of a stimulus[80]. Furthermore, abolishing the oscillatory activity in honey bees through pharmacological manipulation disrupted finer discrimination between odorants[50]. In contrast,
in this work we revealed a non-oscillatory form of neural synchrony observed only at the stimulus onsets and offsets of a novel odorant. We note that these two forms of neural synchrony appear to only have a weak relationship with each other. For example, consistent with earlier work[49, 50], we found that LFP oscillations lasted the entire stimulus presentation duration even after the ensemble spike synchrony was diminished within a second after odor onset. Conversely, LFP oscillatory synchronization was disrupted during stimulus offset but spike synchrony was high during these periods. Lastly, we found that overlapping sequences of stimuli only selectively disrupted ensemble spike synchrony, whereas the LFP oscillatory synchrony persisted (Fig. 4.9; although the power in the gamma band reduced when spike synchrony was diminished for hex(2oct)). Integrating these results with our behavioral results we conclude that the behavioral response predictability correlated better with the non-oscillatory form of ensemble spike synchrony observed at odor onsets and offsets.
Figure 4.9 Odor-evoked oscillatory synchronization in the antennal lobe. (a) Top panel, representative field potential traces (filtered between 10 – 50 Hz) obtained by placing electrodes deep in the mushroom body are shown for four different odors. Bottom panel, spectrogram showing temporal evolution of power in different oscillatory frequencies at the same time scale as top panel. Note, spectrograms obtained for each trial was normalized and averaged across trials and experiments. (b) Similar plots as shown in panel a, but for overlapping odor presentations. Arrows indicate increases in oscillatory power at higher frequencies following the onset of the second odor in the sequence. Note that the oscillatory synchronization persists during the entire duration of the two odor sequences. (c) Average cross-correlogram (250 ms non-overlapping window, 10 trials) between LFP signals simultaneously acquired from two electrodes during different two-odor sequences. The oscillatory LFP signals obtained from different electrodes were highly coherent.

What are the possible mechanisms that might underlie the observed cross-adaptation between odorants? It is possible that some of our results may be explained by adaptation at the
level of sensory neurons themselves. To investigate this issue, we analyzed the sensory neuron responses to overlapping sequences of odorants. We found that the sensory neuron responses to the second odorant pulse reduced significantly only when the two pulses belonged to the same odorant (i.e. hex(hex) case; Fig. 4.10). When two different odorants were presented in a sequence, the response following the introduction of the second odorant was mostly comparable to those observed during its solitary presentation. Therefore, we speculate that dependence on stimulus history can arise either through reduction in synaptic efficacy between sensory neurons and their downstream projection neurons or by enhancing the inhibition to the co-active group of projection neurons by facilitating GABAergic inhibition through local neurons in a heterogeneous fashion.

**Figure 4.10 ORN responses to different stimulus conditions.** Sensory neuron responses averaged across neurons and trials are shown for solitary (red) and overlapping (black) presentations of odor pulses. A significant reduction in peak firing rate was observed only for the second pulse of hexanol (paired t-tests; * $P = 7.5 \times 10^{-5}$. $n = 5$ trials).
We found that the both the overall combination of projection neurons activated as well as their activation profiles were relatively consistent across trials and across different presentation conditions (solitary vs. overlapping), but varied considerably between odorants (Fig. 4.1g). The temporal structure of responses, on the other hand, could vary depending on the presentation conditions (Fig. 4.1e). Based on these, two orthogonal predictions could be made. First, if the fidelity of the ensemble responses’ temporal structure were important, then one possible outcome is that locusts will not recognize a trained odorant when it is presented in sequence following a ‘similar’ odorant (here similarity refers purely to neural response overlaps, for example, hexanol and 2octanol are similar odors). Alternately, if the combinations of neurons activated alone were sufficient for odor recognition, then we would expect trained locusts to recognize a conditioned stimulus irrespective of whether it was presented solitarily or in a sequence. Our behavioral results are consistent with the latter hypothesis that a consistent combinatorial code may be sufficient for recognition of trained odorants by locusts (refer Fig. 4.7a).

Interestingly, we found that the combinations of neurons activated by citral could change significantly depending on whether it was presented solitarily or in a sequence following geraniol (cit vs. cit(ger) case; Fig. 4.1g). This can be clearly seen in the mismatch between neural response trajectories traced by solitary and overlapping introductions of citral and further quantified in the classification analysis (Fig. 4.5). Consistent with these results, in our earlier work, we found that although solitary introduction of citral innately repelled locusts in a T-maze assay, when presented following geraniol with a 2 s lag, citral introductions failed to repel them from the arm delivering this overlapping odor sequence. Hence, these results suggest that odorant identity is altered when the combinatorial code is altered.
We found several parallels between the temporal features of odor-evoked neural and behavioral responses. Consistent with previous works[66-68, 125], we found that odor-evoked responses were stronger and more dynamic immediately following odor onsets and offsets. In between these transient epochs of neural activity, a persistent stimulus could only evoke neural activity patterns that were less intense but more stable. Our dimensionality reduction and classification analyses results revealed that although the activity returned close to baseline levels during these steady-state epochs (causing a reduction in Euclidean distances between ensemble responses[66]), the high-dimensional response vectors were still aligned with those observed immediately following odor onsets (smaller angular distances between response vectors observed over time). Similarly, we found that the palp-opening responses of trained locusts to conditioned stimuli were rapid (median response latency 600 - 700 ms) and persisted as long as the overall population response in the antennal lobe was above the baseline level i.e. during both transient and steady state periods. Furthermore, the behavioral response dynamics, albeit slightly delayed, still matched the ensemble neural response dynamics closely (Fig. 4.7b). Finally, the behavioral responses across locusts were predictable only during those temporal epochs when the neural activities were highly synchronous.

Notably, we found that the behavioral consequence of disrupting synchrony was not in eliminating the responses altogether but in making them highly variable. While a piecewise, high-threshold decoder would be appropriate for interpreting synchronous inputs, decoding an asynchronous response would involve a temporal integrator. Our results suggest that the circuits downstream to the insect antennal lobe may have the ability to interpret different modes of neural transmissions. It appears that temporal integration may happen as long as asynchronous
ensemble neural activities still encode for the identity of the odorant i.e. neural responses trace trajectories within the ‘attractor’ (or ‘sub-space’) that encodes the stimulus identity.

4.4 Author Contributions

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B.R. conceived the study and designed the experiments. D.S., C.L. and N.K. performed the electrophysiological recordings. S.P. did the behavioral experiments. W. P. developed the palp-tracking algorithm. C.L. and D.S. analyzed the data. B.R. and C.L. performed the modeling work. B.R. wrote the paper, and D.S., C.L., N.K. and W.P. provided feedback on the manuscript.
Chapter 5: Adaptation Produces Efficient Neural Coding for Odorant Intensity

5.1 Introduction

Sensory stimuli often elicit responses that are broadly distributed across a large population of interconnected neurons[12]. These stimulus-evoked population activities are patterned over time in part due to stimulus dynamics themselves [130, 156], and in part due to changes in interactions between individual neurons (and are therefore ‘intrinsic’ to the neural network)[25, 114]. The latter is evident in the neural response variations observed over repeated presentations of the same stimulus[85]. While a great deal of work has been done to study the role of stimulus dynamics on shaping neural activity [75, 124, 130], relatively less is understood about the contributions of intrinsic neural network dynamics in sensory information processing[25, 80]. A fundamental problem in systems neuroscience is to determine how neural representations are altered due to relatively slower changes in stimulus-evoked activity observed across trials.

Trial-to-trial neural response changes have often been studied at the level of individual neurons [90, 111, 157]. A standard approach is to regard these trial-to-trial variations as ‘noise’ from which the stimulus-related signals must be extracted[86, 87]. Hence, neural activity is averaged across trials to achieve better a signal-to-noise ratio. In a direct contrast to this approach, a few studies have shown evidence to suggest that changes across trials are not random but a processing feature of neuronal networks [80, 158-160]. If the latter hypothesis is indeed true, what are the functional implications of such changes at the population level and how to quantify them systematically remains a challenge.
In the insect olfactory system, spiking activity in the first-order projection neurons in the antennal lobe that receive direct sensory input (PNs; analogous to mitral/tufted cells in the vertebrate olfactory bulb) tend to reduce over stimulus repetitions [80]. At the ensemble level, the PN activity has been shown to synchronize in an oscillatory fashion leading to slow build-up of local field potential activity[80]. These observed changes in neural activity have been shown to be more short-term in nature, and can arise due to repeated presentation of the same stimulus.

Although trial-to-trial response dynamics has been well documented, their influence on sensory signal processing remains unclear. Here, we show that principal neuron activity, both at the individual and at the population level, systematically changed over trials in a diverse, non-monotonic manner. Although the overall response intensity, as measured by the total number of spikes elicited by a stimulus reduced, odorant concentration was robustly encoded. Hence, our results and analysis suggests that adaptation can allow neural circuits to efficiently represent information about stimulus intensity with fewer spikes.

5.2 Results

5.2.1 Inter-trial Neural Dynamics are Diverse in Individual PNs

We began by examining how odor-evoked responses in individual PNs changed across twenty-five repeated presentations of the same stimulus (Fig. 5.1a). Two independent datasets were used in this study. In one set of experiments, we recorded the responses of 80 PNs to two odorants at two different concentration levels: hexanol 1% (hex-1%) and 0.1% (hex-0.1%), 2-octanol 1% (2oct-1%) and 0.1% (2oct-0.1%). The second dataset included 81 PNs responding to isoamyl acetate 1% (iaa-1%) and 0.1% (iaa-0.1%), benzaldehyde 1% (bzald-1%) and 0.1% (bzald-0.1%). We found that the odor-evoked neural activity patterns across trials were not a
constant but changed in a systematic manner for individual PNs. We characterized these trial-to-trial changes using the following response parameters (Fig. 5.1a): inhibition duration (i.e., the non-spiking period after the initial peak firing activity; PN1, PN4, PN5), response latency (i.e., the time to reach the peak firing value; PN2, PN9, and PN10), response intensity (i.e., the peaking firing rate; PN2, PN6) and reliability (i.e., the firing pattern consistency across trials; PN3, PN7, PN8). These observed features could exist for any neuron-odor combination in our data.

To systematically quantify the trial-to-trial firing pattern changes, we employed a neighboring-trial similarity metric (see Methods in Chapter 2) [111]. Briefly, to analyze each PN responses, we first counted the number of spikes in 50 ms non-overlapping timebins for each trial. The spike counts during the entire 4-s stimulus presentation generated an 80 dimensional time series of spike counts for a given PN in a single trial. Then we measured the similarity between responses observed in a given trial with the mean responses observed in the trials immediately preceding and following (for the first and the last trial, we used two trials that immediately followed and proceeded, respectively). These correlations were computed for each of the twenty five trials and plotted as a function of trial number (Fig. 2.6a). Overall, this correlation metric resulted in various profiles for a total of 644 neuron-odor combinations.
Figure 5.1 Inter-trial neural dynamics in individual PNs. (a) Responses of 10 representative projection neurons (PNs) to different odors and concentrations are shown as raster plots (25 trials each). Arrows highlight the systematic changes in stimulus-evoked response features: inhibition duration (PN1, PN4, PN5), response latency (PN2, PN9, PN10), response intensity (PN2, PN6) and reliability (PN3, PN7, PN8). The entire four seconds odor pulse duration is shown. (b) Neighboring-trial similarity metric for all neuron-odor pairs are shown after clustering (see Methods in Chapter 2). Note that the neural responses are separated in five distinct clusters based on how the responses change across trials. The correlation metrics for representative neuron shown in a are highlighted in bold. (c) The stacked bar plot summarizes the distribution of clusters for each stimulus condition. No significant change in distribution of number of PNs belonging to each cluster type is observed for different stimulus tested (Two-way ANOVA, p>0.05).
To identify the predominant response motifs that characterize trial-to-trial response changes, we used an unsupervised clustering analysis (see Methods in Chapter 2). We found that neighborhood-correlation patterns for all PN (all PN-odor combinations included) could be categorized into five non-monotonic motifs or clusters (Fig. 5.1b; see Methods in Chapter 2). Furthermore, for all odorants and intensities tested, we found that the distribution of the number of cells belonging to each type of cluster did not change significantly (Two-way ANOVA, p>0.05; Fig. 5.1c). Therefore, we conclude that these systematic changes in PN response were odor-independent, and represented a circuit-level adaptation triggered and sustained by any stimulus.

5.2.2 Systematic Changes in Local Field Potential Oscillations

In addition to the diverse patterns observed in individual neuron-odor pair, we also examined trial-to-trial changes in the oscillatory local field potentials (LFPs) activity. For this analysis, we segmented and examined two distinct response epochs (0 - 500 ms and 2 - 4 s, after odor onset) that exhibited distinct oscillatory features (Fig. 5.2a-c). Consistent with previous study[49, 80], within a short time after odor onset (<500 ms; during on-transient activity), the LFP activity had a power spectrum that peaked and centered around 20 Hz (fast oscillation period; Fig. 5.2b upper panel), and oscillatory power systematically increased over repeated trials (Fig. 5.2c upper panel; repeated measures ANOVA: p = 1.13 × 10^{-10}). Conversely, the later epoch of the LFP signals (2 - 4 s after odor onset; after convergence onto steady-state responses), the power spectrum centered around 10 Hz (slow oscillation; Fig. 5.2b bottom panel), and the oscillatory power decreased systematically over trials during this response window (Fig. 5.2c bottom panel; repeated measures ANOVA: p = 5.48 × 10^{-9}). These systematic changes provide an alternate neural correlate for short-term memory at the circuit level but tend to mask the diversity of changes that happened at the single PN level[49, 80].
5.2.3 Systematic Changes in Population PN Firing Activity

Next, we sought to examine how the ensemble neural activities changed across trials. For each individual trial, we calculated the spike count in 50 ms non-overlapping time bins summed across the entire population of PNs (peri-stimulus time histogram; PSTH). PSTH profiles representing PN firing activities were generated for all 25 repeated trials (Fig. 5.2d). We found that the time to reach the peak firing rate value during an odor presentation was rapid and reliable across trials (0.55 ± 0.01 s after odor onset); however the peak firing rate values themselves gradually reduced (one-way ANOVA, p<0.01) for all stimuli used in the study. Furthermore, for each trial, we examined the total spiking activity over the entire 4 s stimulus presentation across all recorded PNs to assay the total spiking activity elicited in the antennal lobe evoked by a particular stimulus in a given trial. We found that the total number of spikes reduced as a function of trial number and in a monotonic manner (Fig. 5.2e; one-way ANOVA, p<0.01).

How do these systematic changes in spike counts impact olfactory information processing? To understand this, we compared the total spiking activity evoked by the same odorant across two concentrations. We found that after adaptation (i.e. during later trials), the total number of spikes elicited by the odorant delivered at a higher concentration became lower than the pre-adapted response (i.e. during first few trials) elicited by the same stimulus at a lower intensity (Fig. 5.2e). For instance, the number of spike elicited by hex-1% during last 10 trials was fewer than the spike count elicited by hex-0.1% in the first trial. This observation raises the following important question: is the information about odor intensity invariant with respect to adaptation? To examine this issue, we next visualized the spatiotemporal neural activities on a trial-by-trial basis using a dimensionality reduction analysis.
Figure 5.2: Trial-to-trial systematic changes in ensemble neural activities. (a) Local field potential traces evoked by hex-1% (filtered between 5-55 Hz) are shown for a subset of 25 consecutive trials. Two distinct analysis windows (shown on the top of the traces) are identified. Fast oscillation epoch is defined as the time segment during the first 500ms following odor onset, while slow oscillations period refers to the duration after 2s of odor onset. The color box indicates the stimulus presentation. (b) For all individual trials, power spectra were obtained using fast Fourier transformation during the two analysis epochs identified. The dotted line indicates a 20Hz frequency band (10-30Hz for fast oscillations; 5-25Hz for slow oscillations) that was used for further analysis. (c) Standardized power for each trace was calculated by integrating total power over the identified frequency band. To allow comparison, the total power in the frequency band was divided by the mean response across 25 trials. n indicates the number of experiments used in the analysis. A repeated measure one-way ANOVA was used for testing the significance of the reported results. (d) Summed stimulus-evoked spiking activities across neurons are shown as a function of time (x-axis) and trials (y-axis). Note that the spike counts are shown on logarithmic scale to only focus on prominent trends and to allow comparison across stimulus presentations. The gray bar along the x-axis indicates the stimulus duration (4s). The upper and bottom panels show the neural activities evoked by the same stimulus at a higher (1%) and lower (0.1%)
concentrations, respectively. (e) Summed spike counts during the entire four-second stimulus exposure across all neurons (i.e. integration along x-axis during odor presentation period) were calculated and plotted as a function of the trial number. The dashed line indicates the minimum spike count observed in twenty-fifth trial for high stimulus intensity odor exposures. A two-way ANOVA was used to compare the spike counts between different trials and different odorant concentrations.

**5.2.4 Visualization of Ensemble Neural Activities on a Trial-to-trial Basis**

How do the odor-evoked spatiotemporal neural responses change across trials? To understand this, we began by analyzing a small dataset consisting of 11 simultaneously recorded PNs from a single locust’s antennal lobe. Two odors, iaa-1% and bzald-1% were delivered for 4 s stimulus pulses over 25 repeated trials. The raw dataset obtained from this experiment was comprised of the following three dimensions: 11 neurons x 80 time bins (4 s with non-overlapping 50 ms bins) x 50 trials (25 trials for each odor).

To visualize this high dimensional neural activity, we first directly performed a linear principal component analysis (PCA). To perform PCA, the three-dimensional neural data cube (neuron x time x trial dimensions) was first unfolded into a two-dimensional matrix by concatenating response across trials (matricization; 11 neurons by 4000 time bins [80 time bin per trial x 50 trials]). This high-dimensional data was subsequently projected onto the first three eigenvectors of the covariance matrix for visualization (see Methods of Chapter 2; results in a 3 by 4000 matrix to facilitate visualization). The ensemble responses in consecutive 50 ms time bins were linked in temporal order and low-pass filtered to obtain individual trial-by-trial odor trajectories shown in Fig. 5.3d. Note that each trial generated a single loop response trajectory after dimensionality reduction. Twenty-five such trajectories (shown in red) correspond to the responses evoked by iaa in each of the twenty-five repeated trials. Similarly, twenty-five bzald trajectories (shown in blue) are also shown for comparison. Here the distinct shades of red and
blue are used to indicate representative trials (1, 5, 10, 15, 20, and 25). We found that the response trajectories varied across trials but still formed two distinct response clusters based on their odor identities. However, the systematic changes across trials observed in ensemble spike counts were not revealed by this analysis. This we note is due to the larger number of free parameters used for his unfold first then perform PCA-based dimensionality reduction techniques that result in a model with larger degrees of freedom (more parameters) to approximate the dataset. For example, an \( n \)-factor principal component analysis (PCA) model of a multi-unit dataset with \( N \) neurons \( \times \) \( T \) time bins \( \times \) \( S \) stimulus would require \( n \times T \times S \) scores and \( n \times N \) loadings. These additional degrees of freedom cause this decomposition of the original dataset to overfit and capture the noise in this dataset and thereby can mask important latent structures.

Therefore, to understand how ensemble activities change across trials, we analyzed the same dataset using a direct tensor-based decomposition method (Fig. 2.3a; see Methods of Chapter 2) [109]. First, we approximated the three-dimensional tensor directly as a sum of the outer-product of multiple rank-1 tensors: \( a_{if} \) (neuron dimension), \( b_{jf} \) (time dimension), and \( c_{kf} \) (trial dimension). The component vectors were found using an alternating least square to minimize the mean-squared reconstruction error. Therefore, the rank of the reconstructed tensor could be utmost equal to the number of factors used for reconstruction. The number of factors itself was a free parameter that was determined using a core-consistency diagnostics (Fig. 5.3b; see Methods in Chapter 2) [110]. Subsequently, the reconstructed tensor was unfolded as before and a PCA dimensionality reduction was performed to visualize the odor-evoked response trajectories on a trial-by-trial basis(Fig. 2.3b).
Figure 5.3 Visualization of ensemble neural activities on a trial-to-trial basis. (a) Peristimulus-time histograms (PSTH) are shown for 11 simultaneously collected PNs from a single locust antennal lobe. Responses to two odorants at a single concentration are shown: iaa (1% v/v dilution) and bzald (1% v/v dilution). Only firing rate responses during the four seconds of odor pulse duration are shown for each neuron. (b) A core consistency diagnostics was used to determine the number of factors to be used in the tensor-based decomposition analysis. Here, the core consistency metric was above 80% for up to three factors decompositions, but fell below 40% when the factor number was increased to four or greater. Therefore, a three-factor decomposition was used for further dimensionality reduction analysis. (c,d) Odor-evoked ensemble neural spiking activities from a single locust after dimensionality reduction using principle component analysis are shown (PCA; see Methods of Chapter 2). Each axis corresponds to one of the first three principal components that capture a certain amount of variance in the dataset. The odor
trajectories generated by iaa and bzald are shown in blue and red, respectively for all 25 trials. For those of trial 1, 5, 10, 15, 20, and 25, neural trajectories are highlighted with color gradient that goes from light to dark. All trajectories start from a pre-stimulus baseline that is indicated as B in the plot. Arrows indicate the direction of response evolution over time. The PCA following the tensor-based decomposition method were used to generate odor trajectories in panels e, while a direct unfold-then-PCA approach was used to generate results in panel d. (e,f,g) Loading vectors obtained from the tensor-based decomposition corresponding to the neuron (e), time (f), and trial (g) dimensions are shown.

We found that the tensor-based decomposition method yielded odor-evoked response trajectories that captured the inter-trial dynamics better than direct-PCA (Fig. 5.3c vs. Fig. 5.3d). As can be noted, the ensemble activity changed systematically across trials. The response trajectories showed a systematic change from light colors (early trials) to darker colors (late trials). Notably, the population responses changed such that the trajectories evolved in similar direction, but the length of the trajectory monotonically reduced over repeated trials. It is worth to note that these changes are akin to those produced by reduction in odor concentrations [69].

Furthermore, we found that the loading vectors obtained by tensor-based decomposition along neuron, time, and trial dimensions represented the underlying structure in the dataset. For example, the loading vectors along neuron dimension represented the neural tuning to the two odorants presented (Fig. 5.3e). For example, neuron-factor1 represented iaa-response selectivity across neurons. PN1, PN3, PN4, PN5 and PN6 have strong response to iaa and therefore larger loading values. In comparison, neuron-factor3 represented bzald-tuning across neurons (PN2, PN3, PN5, PN6 have larger values in Fig. 5.3a, e). On the other hand, neuron-factor2 was indicative cells that responded to both odorants (e.g. PN3, PN5).

Similarly, the loading vectors along time mode provided insights into temporal response motifs (Fig. 5.3f): (i) excitation (indicated by time-factor1; Fig. 5.3f blue trace), (ii) inhibition switching to excitation (indicated by time-factor 2; Fig. 5.3f green trace), and (iii) inhibition
throughout the entire stimulus duration (indicated by time-factor 3; Fig. 5.3f red trace). All of these temporal profiles could be identified in the individual cell response profiles (PSTH profiles in Fig. 5.3a).

Notably, the loading vectors along trial dimension represented the identity of the stimulus used in each trial (Fig. 5.3g). Note that this dataset was comprised of 50 trials, with the first set of 25 iaa trials, and the second-set of 25 bzald trials. Consistent with this dataset organization, the loadings vectors along trial-mode also revealed that there were two distinct combinations of loading values along the trial-dimension. Note that the first set of twenty-five trials had a distinct set values compared to the second set thereby indicating that two different odorants were used in these trials. Therefore, these results indicate that direct decomposition of high-dimensional neural datasets is an effective approach for revealing latent structures in ensemble neural activities.

We took advantage of this decomposition technique and applied it to two larger datasets, one examining responses of 80 PNs to hex-2oct odor pair and the other set examining responses of 81 PNs responding to iaa-bzald. These multi-unit recordings resulted in three-dimensional data cubes that were first decomposed into a sum of rank-1 tensors, and then approximated by sum of the outer product of the loading vectors. Subsequently, the reconstructed three-way data array was unfolded and analyzed by PCA to generate odor trajectories (Fig. 5.4a,b). Each trial of ensemble neural activity formed a single response loop after dimensionality reduction. Note that same trajectory results were shown from two different views to allow better comparison across conditions (Fig. 5.4a,b left and right panel). Similar to the results obtained in the smaller dataset, the ensemble activity changed systematically with trials showing a systematic shift from light
colors (early trials) to darker colors (late trials), indicating that the adaptation altered the overall representation of a particular stimulus. However, these changes did not alter the direction in which these trajectories evolved over time, indicating that certain neural response features may robustly represent information about odor identity and intensity. More importantly, closer examination of the loading vectors obtained along the trial-dimension clustered based on odor identity followed by intensity (Fig. 5.4c,d). Hence, these results suggest that ensemble neural activity might robustly encode information about both odor intensity and identity even though the total number of spikes may change substantially with adaptation.

Figure 5.4 Trial-to-trial ensemble neural dynamics. (a,b) Similar odor trajectory analysis as in Fig. 5.3c but for a complete dataset that contains all the recorded neurons pooled across experiments. For each odor pair (panel a for hex-2oct; panel b for iaa-bzald), two different perspectives are shown to better reveal the underlying response patterns. B indicates the time the stimulus onset. Arrows indicate the direction of response’s evolution over time. The response trajectories of representative trials 1, 5, 10, 15, 20 and 25 are highlighted by color gradient from light to dark. (c,d) The loading elements along the trial mode obtained by tensor decomposition are plotted in a three-dimension space for hex-2oct (panel c) and iaa-bzald (panel d). Color gradient from light to dark corresponds to the increasing number of trials. The solid circle and star symbols represent the higher (1%) and lower (0.1%) odorant concentrations, respectively.
5.2.5 Combinatorial Code is Adaptation-Invariant

To further quantify these observations, we summed the number of spikes over the entire odor exposure period (4 s) for each individual PN and compared the projection neurons response profiles generated by two different odorants or by the same odorant at two different intensities (Fig. 5.5 a,b). We used a correlation-based response metric to quantify the similarity between two population response profiles. We found that the combinatorial PN activity profiles varied substantially between odorants and subtly between different concentrations of the same odor, (Fig. 5.5a; $R < 0.4$, for hex vs. 2oct, and iaa vs. bzald vs. $R = 0.692$ for hex-1% vs. hex-0.1%; $R = 0.467$ for iaa-1% vs. iaa-0.1%). These results suggest that a combinatorial code may be a robust way to represent both odor identity and intensity.

Further, we examined the effect of adaptation on the combinatorial PN response profiles (Fig. 5.5b). We found that the correlation between response profiles changed systematically as a function of trial number (note that similarity between response profiles observed in a given trial with the first trial of the same stimulus is shown). However, these changes were subtle when compared with the changes due stimulus intensity and identity. Therefore, these results suggest that the stimulus information, including both odor identity and intensity, can be robustly encoded by the combinations of neurons activated even in presence of neural adaptation.

Note that, the results in Fig. 5.5b were obtained based on making comparison with the responses obtained in the very first trial. To generalize our conclusion, we performed a hierarchical clustering analysis of these combinatorial profiles (a correlation distance metric was used, see Methods; Fig. 5.5c). We found that the ensemble response profiles were grouped first based on odor identity followed by intensity. Moreover, within each cluster of the same stimulus
(indicated by the same color), the leaf nodes of the dendrogram tended to form sub-groups based on the trial sequence. For example, early trials seemed to aggregate together as a distinct sub-group compared to the later trials. Therefore, we conclude that the systematic changes within each stimulus condition only slightly perturbed but did not significantly alter the overall combinatorial profiles. In sum, our results reveal that a combinatorial code is adaptation-invariant and could robustly represent both the identity and intensity of a stimulus. Since, the same information about a stimulus is represented with fewer spikes in the later trials, we conclude that adaptation refines the odor codes by making them more efficient.
Figure 5.5 Inter-trial dynamics efficiently encode stimulus intensity. (a) Summed spike counts over the entire duration of odor presentation (4 s) are shown for all PNs (mean ± S.D.; n = 25 trials). PNs are sorted based on their response to one of the two stimulus compared. Correlation coefficient ($R$) calculated between PN response profiles to two odorants compared is shown in each panel. (b) The similarity between the PN ensemble response patterns is shown as a function of trial. Similarity is compared with respect to the responses observed in the very first trial. Open circle denotes comparisons between different trials of the same stimulus, other symbols indicate comparisons between different stimuli or between the same stimulus at different intensities. Note all comparisons are made with respect to the first.
trial of hexanol at 1%. Top panel: comparison between hex-1% with hex-0.1%, 2oct-1% and 2oct-0.1%. Bottom panel: comparison between iaa-1% with iaa-0.1%, bzald-1% and bzald-0.1%. (e) Results from a hierarchical clustering analysis are shown. Dendrogram was generated using a correlation distance metric comparing trial-by-trial ensemble spiking activities evoked by two different stimuli at two different intensities. Two major response clusters that correspond to stimulus identity and intensity can be noted. The number at the leaf node represents the trial number. Same color convention used as in Fig. 5.2e.

5.3 Discussion

We examined how stimulus identity and intensity can be represented by neural activation profiles and whether the inter-trial dynamics can confound the information encoded by a neural ensemble. We found that the ensemble PN activity reduced upon repeated exposures to a particular stimulus. As a consequence, the number of spikes elicited during later trials of an odorant at a higher intensity became comparable to responses elicited during early trials of the same stimulus at a lower intensity. Our analysis results revealed that this adaptation only altered the number of spikes, but not the combinatorial neural profile for a stimulus. Therefore, these results reveal that the inter-trial dynamics refined neural activities and efficiently encoded the same information about stimulus identity and intensity with fewer spikes.

We visualized the spatiotemporal neural activity in the antennal lobe using a direct dimensionality reduction technique (e.g. PCA). While it has been well-suited to qualitatively describe ensemble PN activity averaged across trials, this technique was inadequate in capturing the systematic changes that happened on a trial-by-trial basis. To overcome this issue, we used a direct high-dimensional data decomposition technique. The better performance of the tensor-based factoring approach used over PCA was due to fewer degree of freedom (number of parameters) used to model the data. For example, for a dataset comprised of 11 neurons, 80 samples (4 s duration; 50 ms binsize) and 50 trials (shown in Fig. 5.3), PCA model with three components requires 12033 parameters including 12000 scores (3 factors x 80 samples x 50 trials
) and 33 loadings (3 factors \( \times \) 11 neurons). In contrast, for the same dataset, tensor-based decomposition only requires 423 parameters [for each factor: an 11 dimensional vector along neuron dimension + an 80 dimensional vector along time dimension + a 50 dimensional vector along trial dimension; a 3-factor decomposition was used in all our analyses]. We note that this analytical technique not only captured the trial-by-trial variation in neural responses, but it also provided meaningful decomposition of the dataset.

Are these trial-to-trial changes observed in PN firing activity a feature of odor processing central circuits or a trivial result produced due to experimental limitations such as electrode drift? To determine this, we repeated the same stimulus presentation (hex-1% and iaa-1%) and recorded the neural activity from the same set of neurons after a sufficiently long inter-block interval (>15 minutes). Previous studies have shown that antennal lobe circuits return to their naïve state when a fifteen-minute interval is used between olfactory stimulation. Consistent with these findings, we found that the systematic trial-to-trial changes were repeatable in individual (Fig. 5.6a) and ensemble PN activities (Fig. 5.6b). This interpretation was confirmed with the dimensionality reduction analysis that revealed that ensemble PN responses were highly reproducible (Fig. 5.6b vs. 5.4c,d). These qualitative results were further confirmed using a quantitative similarity comparison across trials (trial-by-trial response similarity plot shown in Fig. 5.6b). As can be noted, the similarity between response profiles (inverse of distance values) systematically changed over trials.
Figure 5.6 Trial-by-trial response changes are reproducible. (a) Raster plots of four representative PNs responses to two sets of identical stimulus are shown. The same stimulus used was delivered again after a no olfactory stimulation period of greater than 15 minutes. The color box represents 4-s stimulus duration, and the arrows in the box indicate the systematic change in individual PN responses. (b) Similar plots as in Fig. 5.4c,d are now shown for the repeated block of twenty-five trials for each stimulus used in our study. The similarity (inverse of Euclidean distance) between trial mode symbols is shown as a function of trial. Similarity is compared within the same stimulus condition with respect to the responses observed in the very first trial. (c) Similar plot as in panel b, but now shown for a trial-shuffled dataset. Systematic changes over trials are no longer evident here.

Are the systematic changes observed in ensemble PN activity captured faithfully by the analysis technique used? To understand this, we compared the response similarity across trials using the high-dimensional dataset and in the three-dimensional space spanned by the three loading vectors in the trial dimension (i.e. after dimensionality reduction). As can be noted in
Fig. 5.5b and 5.6b, we found that the changes captured in both these analyses were essentially identical. Furthermore, we repeated this analysis by randomly shuffling the trial number associated with each ensemble neural responses. As can be expected, we no longer found any systematic changes across trials after trail-shuffling (Fig. 5.6c). Hence, we conclude that systematic changes observed in ensemble PN activity is an underlying feature of the antennal lobe network and induced by robust and repeatable inter-trial dynamics.
Chapter 6: Conclusion

The main objective of this work has been to determine what roles neural response dynamics play in olfactory information processing and how it influences behavioral responses. In particular, we focused on examining the relationships between different phases of temporally patterned neural responses and behavioral output during those epochs.

First, we examined how transient reorganization of neural responses allowed the antennal lobe neural circuit to track and represent a new stimulus in presence of a background odor. By presenting pairs of odorants simultaneously or in overlapping sequences, we found that a few hundred milliseconds was sufficient for the neural circuits to create response dynamic transition, which resulted in a response pattern match of odor-evoked responses across different stimulus presentations. Can this pattern match observed in physiology be used to predict behavioral recognition of odors? To answer this, we assessed the recognition performance of locusts using an appetitive-conditioning paradigm. Note that, although locusts have been a popular model for studying neural representations of odors, the lack of a behavioral assay to probe the relevance of neural activity to the organism has severely impeded further progress. Here, our work is the first such effort to bridge this gap. Under identical stimulation conditions with our physiological experiments, our behavioral results suggested that any partial pattern match of an odor across conditions was sufficient for robust odor recognition. Therefore, we identified a neural basis for background-invariant odor recognition.

However, we found that odor recognition could not be achieved for an odor pair, citral—geraniol. In this case, neural activity did not achieve pattern match across conditions. Furthermore, we found that binary mixtures of citral and geraniol, and overlapping sequences of
these odorants that evoked responses more similar to solitary geraniol exposures. Interestingly, our behavioral study using a T-maze assay revealed that, citral served as a repellent to locusts whereas geraniol acted as an attractant. Our results indicated that an overlapping sequence of geraniol and citral also attracted locusts in the T-maze assay, consistent with predictions from our physiology data. Taken together, these results reveal that both innate and acquired behavior could be predicted based on the neural response patterns observed in the antennal lobe.

Note that, this dynamic reorganization of neural responses predominantly occurred during the on-transient state. What then is the importance of neural response during the steady state that followed the on-transient state? Our results suggested that neural activity during this dynamic epoch selectively interfered with responses elicited by an ensuing odorant. Moreover, this interference perturbed certain temporal features of neural activity (such as coincident spiking across the neural ensembles), but did not alter the combination of neurons activated. Various degrees of interference were observed for different odor combinations, which were determined by the similarity between two odors presented in the sequence.

How those temporal and spatial response profiles observed in the antennal lobe impact behavioral outcomes? To answer this, we needed a quantitative approach for our behavioral study. Here we used an image-processing algorithm to track movement of locust maxillary palps, which allowed us to record behavioral responses with very fine temporal resolution (100 ms), and further make fine-grained comparisons between our physiology and behavioral results. Our results revealed that the spatial profiles of neural activity that were well maintained across conditions were sufficient to allow locusts to robustly recognize a trained odor presented in both solitary and overlapping fashion. Moreover, the changes in temporal profile of a neural response did not eliminate the behavioral responses, but made them highly variable or unpredictable.
Notably, the behavioral responses across locusts were predictable only during those epochs in which the temporal structure (neural synchrony) was maintained. Hence, this work reveals how spatial (odor identity) and temporal (odor novelty) features of ensemble neural response translate to shape stimulus-evoked behavioral responses.

We also examined stimulus-evoked neural dynamics on a longer timescale across repeated trials (i.e. inter-trial dynamics). Here, we sought to explore the role of this inter-trial dynamic in olfactory information processing. We found that inter-trial dynamics could lead to systematic suppression of neural activity (reduction in total spike count) in the antennal lobe, through a circuit-level adaptation process. This adaptation appeared to hinder the antennal lobe circuit’s ability to faithfully encoding information about odor intensity, since it made spiking activities variable across trials and comparable across two different concentrations for the same odor. However, using a novel trial-by-trial analysis of ensemble neural activity, we found that odor information, including both identity and intensity, could be robustly maintained even in presence of the circuit-level adaptation. Hence, the adaptation could refine neural activities and efficiently encode the same information with fewer spikes.

Additionally, the novel technique we used for analyzing multi-unit neural activity patterns, provided insights into latent structures of the high-dimensional dataset. Further, since our analytical technique required fewer parameters, it was easier to interpret than conventional dimensionality reduction approaches like PCA and LLE.

In sum, this thesis work provides fundamental insights regarding behaviorally important features of olfactory signal processing in a relatively simple biological olfactory system.
References


