Hierarchical excitatory synaptic connectivity in mouse olfactory cortex

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Topological motifs in synaptic connectivity—such as the cortical column—are fundamental to processing of information in cortical structures. However, the mesoscale topology of cortical networks beyond columns remains largely unknown. In the olfactory cortex, which lacks an obvious columnar structure, sensory-evoked patterns of activity have failed to reveal organizational principles of the network and its structure has been considered to be random. We probed the excitatory network in the mouse olfactory cortex using variance analysis of paired whole-cell recording in olfactory cortex slices. On a given trial, triggered network-wide bursts in disinhibited slices had remarkably similar time courses in widely separated and randomly selected cell pairs of pyramidal neurons despite significant trial-to-trial variability within each neuron. Simulated excitatory network models with random topologies only partially reproduced the experimental burst-variance patterns. Network models with local (columnar) or distributed subnetworks, which have been predicted as the basis of encoding odor objects, were also inconsistent with the experimental data, showing greater variability between cells than across trials. Rather, network models with power-law and especially hierarchical connectivity showed the best fit. Our results suggest that distributed subnetworks are weak or absent in the olfactory cortex, whereas a hierarchical excitatory topology may predominate. A hierarchical excitatory network organization likely underlies burst generation in this epileptogenic region, and may also shape processing of sensory information in the olfactory cortex.

piriform cortex | endopiriform nucleus | small world | functional connectome | network topology

Structural and functional plasticity at excitatory synapses in cortical networks represents a fundamental mechanism for encoding sensory representations and memory. As a result, neuronal ensembles that are connected with high probability emerge as functional units to produce a population code of the environment. The topology of such excitatory circuits should contain signatures—as global topological motifs—that reflect the encoding strategy. The cortical column is a well-studied example of such a motif (1). Columnar cortices contain substantial distributed connectivity and some brain areas, such as association cortex, high-order cortices, and the piriform cortex, lack a pronounced columnar structure. In the piriform (olfactory) cortex, there exists only a rudimentary understanding of the relationship between network structure and cortical function. The axons of individual piriform pyramidal neurons ramify widely throughout the olfactory cortex, and only show patchiness on a very broad scale (2–4). Consistent with this architecture, neural activity in response to individual odorants is distributed broadly across the olfactory cortex as detected by 2-deoxyglucose, c-fos expression, multiunit recording, and population calcium imaging (5–8). Likewise, the receptive fields of individual neurons in piriform cortex and anterior olfactory cortex are broad (9, 10). Broad receptive fields in piriform cortex reflect convergence of input from many olfactory bulb glomeruli (11) and are strongly influenced by recurrent connectivity (12).

These observations support a highly distributed population representation but reveal little about what processing function

the piriform cortex performs. Physiological and anatomical studies have provided some clues. For example, neuronal responses in piriform cortex are specific for category of odorant (13), and odor identity and similarity are separately encoded in anterior and posterior piriform cortex, respectively (14), suggesting hierarchical coding. The endopiriform (EN) and preendopiriform nucleus (pEN), immediately adjacent to the piriform cortex, have dense recurrent connectivity and dense connectivity with overlying areas of piriform cortex (15, 16). The pEN, also called area tempestas, is a highly epileptogenic locus (16, 17). However, the physiological role of its dense connectivity is unknown (15, 18).

To probe excitatory connectivity in the olfactory cortex, we isolated excitatory synaptic activity in a tailored brain slice containing the ventral anterior piriform cortex (APC_V), the pEN, the anterior olfactory cortex (AOC; also called anterior olfactory nucleus). Using weak stimulation of the lateral olfactory tract (LOT) input while blocking GABAergic inhibition and NMDA receptors, we evoked transient, all-or-none, network-wide bursts of excitation. Network-wide transient bursts are a dynamic circuit property shared by the hippocampus, neocortex, and piriform cortex in disinhibited recording conditions (19–21). We used the pairwise variance patterns detectable in the fine structure of these bursts as a probe of excitatory network topology. We compared whole-cell recordings from randomly selected pairs of principal neurons in olfactory cortex with patterns generated in simulated networks with a range of network topologies. Our findings suggest that excitatory connectivity in olfactory cortex is neither random nor organized into local or distributed subnetworks. Rather, it shows hierarchical connectivity.

Significance

The dynamics of activity in neural networks form the basis of many brain functions ranging from neural plasticity, memory formation, and sensory perception to consciousness. These dynamics are shaped by immensely complex connectivity patterns of excitatory and inhibitory synapses. Here we used the variance structure of network-wide bursts of excitation to examine connection topology in the olfactory cortex—often viewed as a randomly organized network—and compared it with simulations of several network models. A hierarchical network topology best reproduced the excitatory activity in this cortical region as a result of a subpopulation of neurons (the hierarchy) that generate numerous connections, particularly with members of the same subpopulation. This network organization provides clues to sensory processing in the olfactory cortex.

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Results

Pairwise Variance Analysis of Excitatory Synaptic Bursts. To examine the excitatory connectivity in olfactory cortex, we developed a brain-slice preparation containing the APC_V, subjacent pEN, and the AOC (Fig. 1*A* and *B*). Low-intensity stimulation of the LOT (0.05–0.1 Hz) evoked a burst of inward current in the presence of picrotoxin (50 μ M; Fig. 1*C*) in the AOC, the most rostral subregion of olfactory cortex contacted by axons from the olfactory bulb. The bursts consisted of two components that could be captured by a double exponential fit with decay time constants of 12.6 ± 0.4 and 185 ± 7 ms (Fig. 1*C*, red curve; $n = 9$). To better resolve the timing of fast synaptic events we blocked NMDA receptors using 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) (5 μ M). Under these conditions, single weak shocks to the LOT at the threshold for burst generation (~ 12 V) resulted in a monosynaptic response in most cells

Fig. 1. Network-wide excitatory synaptic bursts in brain slices of olfactory cortex. (A) Image of the right hemisphere of a mouse brain placed on a machined acrylic block (Left). A diagram of the preparation (Right) illustrates the angle of section ($\phi = 15^\circ$, $\phi = 40^\circ$). (B) Montage of DIC images from a typical slice (Upper). Diagram of brain regions present in the slice (Lower). A key for abbreviations can be found in *SI Materials and Methods*. Cortical regions indicated with color were recorded from. (C) Superimposed responses of an AOC neuron to LOT stimulation (30 V): before (black trace), during (gray traces), and after complete (blue trace) wash-in of picrotoxin, at a holding potential of -75 mV. The red curve is a fit with a sum of two exponentials ($\tau_f = 14.3$ ms, $\tau_s = 280$ ms). (Inset) LOT stimulation (7 V) in a different AOC neuron, voltage clamped at -40 mV, showed inward current, followed by outward inhibitory currents, similar to previous results (52). (D) LOT stimulation in the presence of NMDA antagonists still produced all-or-none bursts of the same amplitude (light blue trace), but the duration was decreased (compare with C). Black trace shows a response to stimulation at threshold that failed to elicit a burst. Inset shows a histogram of the burst amplitude at threshold. (E) Four trials of paired burst responses to repeated LOT stimulation (8 V, trial number at left) for a typical paired recording of a neuron in AOC (black traces) and a neuron in APC (red traces).

(Fig. 1*D*, both traces), followed by a burst of excitation with discernible substructure in suprathreshold responses (Fig. 1*D*, blue trace). Bursts occurred in all cells in all subregions of olfactory cortex in response to graded LOT stimulation: AOC ($n = 50$), APC_V ($n = 30$), and pEN ($n = 17$). Bursts were all or none, as indicated by a histogram of burst amplitudes at threshold (Fig. 1*D*, Inset). Burst amplitudes in the AOC were not altered by CPP (control: 491 ± 7 pA; $n = 37$; CPP: 504 ± 5 pA; $n = 9$; $P = 0.78$). Blockade of NMDA receptors revealed a slow outward current that could be blocked by the GABA_B antagonist CGP55845 (10 μ M; ref. 22). Thus, picrotoxin and CPP were included in subsequent experiments to examine bursts generated by fast excitatory synaptic activity.

To analyze the network response, we made paired recordings of pyramidal neurons selected at random and separated from each other in most cases by large distances in the slice (up to 3 mm). An example recording configuration is shown in Fig. 1*B*, Bottom. We assessed the bursts in response to repeated trials of weak LOT stimulation at the same intensity. In a given cell, the shape of each burst varied significantly across trials (Fig. 1*E*, compare intertrial sets of black or red traces). However, burst waveforms were remarkably similar between pairs of neurons for each trial (Fig. 1*E*, compare intratrial pairs of black and red traces), despite their random selection and the large distance between them (see also Fig. S1). To quantify the intratrial similarity, we measured the coefficient of variation (CV) of the burst waveforms between cells across trials (intratrial) as well as across trials within each of the cells (intertrial). Because the latency to bursts varied significantly between trials (Fig. 1*E*), we first corrected for latency. An analysis of burst latencies and the timing of bursts within subregions of the olfactory cortex revealed that bursts in some subregions lagged others by up to 10 ms ($F_{3,30} = 5.2$, $P < 0.005$; Fig. 2; Fig. S2). Network bursts in AOC neurons lagged behind the piriform network, and in particular behind APC_{V-R}. Because of these lags between cortical subregions, the burst CVs were compared for a range of lags, similar to a cross-correlation, and the minimum coefficient of variation as a function of lag (CV_{lag} ; called cross-variation), was determined. For paired recordings between AOC and APC, the intratrial minimum cross-variation between cells (CV_{pair}) was 0.57 ± 0.12 , which was significantly smaller than the within-cell intertrial cross-variation (CV_{cell}) of 0.72 ± 0.09 ($n = 8$ pairs; $P < 0.05$).

Synaptic Bursts of Excitation in Cortical Network Models. Because burst waveforms were surprisingly similar on a given trial across large distances in the olfactory cortex, we wondered what topological features of the excitatory network were responsible. To address this issue, we created large-scale network models of excitatory neurons with realistic intrinsic membrane properties, local short-term synaptic plasticity, and conduction delays (refs. 22, 23; *Materials and Methods*; Fig. S3*A*). Substantial anatomical and physiological evidence support a highly distributed, and possibly random, organization of excitatory connections in olfactory cortex (2, 3, 5–10). Thus, we first generated network models with randomly distributed connectivity between neurons, organized in a line representing the long axis of the ribbon-shaped piriform cortex. Stimulation of random networks generated bursts that were all or none and highly synchronous across neurons, as shown by a color map of the burst conductance as a function of time in each of the 6,400 neurons (Fig. 3*A*, Top), consistent with previous observations (24–26). For the random network, the intratrial variation in burst waveforms ($CV_{\text{pair}} = 0.36 \pm 0.05$) was less than the intertrial variation for a given cell ($CV_{\text{cell}} = 0.41 \pm 0.04$; $P < 0.001$; Fig. 3*B*). However, the extent of the difference ($CV_{\text{pair}} - CV_{\text{cell}}$) was threefold smaller in the random network models (-0.050 ± 0.048) than in the experimental data (-0.15 ± 0.17 ; $P < 0.05$; Fig. 3*D*). Thus, we

Fig. 2. Timing relationships between subregions of the olfactory cortex. (A) Example traces from a neuron in AOC (Upper, black) and a neuron in APCV-R (Lower, red). Vertical dashed lines indicate the window used for cross-correlation measurements, which begins after the monosynaptic current and ends following the burst current. Longer segments were used for some pairs to accommodate variability in onset latency. (B) The cross-correlation of the traces in A exhibited a single peak at a lag of 10.6 ms, corresponding to later bursts in the AOC neuron. (C) Lag, measured from the maximum cross-correlation, was plotted for paired recordings between a neuron in AOC and a neuron in a second location in the slice (x axis). AOC 'c' is the more caudally located neuron (closer to piriform) in paired recordings of two AOC neurons.

examined other network models to determine what topological features would better fit the experimental data.

Many cortical networks are organized into columns with some long-range connections, referred to as small-world networks. In contrast to random networks, small-world networks exhibited bursts that evolved somewhat independently within each column and thus appear as clusters (Fig. 3A, Bottom). In the example shown, there were 32 columns with 200 neurons each. Thus, unlike recordings in piriform cortex burst waveforms in small-network models were more similar within a column (intertrial comparison, Fig. 3C) than across columns (intratrial comparison, Fig. 3C). For networks with strongly columnar topology (70–80% within column connections), $\overline{CV}_{pair} - \overline{CV}_{cell}$ was quite positive [0.12 ± 0.15 (80%)]]; whereas weakly columnar networks (20% within column connections) had a $\overline{CV}_{pair} - \overline{CV}_{cell} = -0.04 \pm 0.01$, comparable to random networks. Moderately columnar networks were intermediate (0.02 ± 0.02 for 60% within-column connections; 0.004 ± 0.02 for 40% within-column connections). A summary bar graph (Fig. 3D) and summary scatterplot (Fig. S3B) shows that the within- and between-cell variance patterns in both random and columnar networks were inconsistent with our experimental data (Fig. 3D).

Although there is a lack of evidence for anatomically discrete columns in olfactory cortex, an intriguing possibility remains that groups of neurons interconnected with high probability are distributed diffusely throughout the cortex. Such connectivity could help account for the complexity of odor space and possibly encode odor objects (27). However, distributed subnetworks would be difficult to detect, even with extensive connectomic data. For example, a complete connectome of a network with 1,000 neurons with 10 subnetworks and 50% within subnetwork connections would look completely random when unsorted (Fig. 4A, Top). However, if the subnetwork membership were known, sorting of the connectivity matrix according to subnetwork membership would clearly reveal the presence of distributed

subnetworks (Fig. 4A, Bottom). We hypothesized that distributed subnetworks would disrupt the correlation in fine structure of network-wide synaptic burst waveforms in paired recordings. To test this idea, we created network models with identical connection topology to columnar models, except that subnetwork members were distributed randomly in space throughout the line network model. An example of the traces generated by the distributed subnetwork model is shown in Fig. 4B. Indeed, network models with distributed subnetworks ranging from 20 to 80% within subnetwork connections had comparable within- and between-cell $\overline{CV}$ s (Fig. 4C, red bars), and also failed to match the experimental data (Fig. 4C, blue bar).

Power-Law and Hierarchical Connectivity in Olfactory Cortex. Because the above network models failed to capture our experimental data, we explored other physiologically motivated network structures. Several lines of evidence suggest that the olfactory cortex processes information in a hierarchical manner (13, 14, 28, 29). The EN and pEN, subjacent to the piriform cortex, give rise to extensive excitatory connections that could serve as a substrate for hierarchical connectivity within the olfactory cortex (15, 16). However, the precise connectivity of EN and pEN with the piriform cortex is not known. We considered two connectivity scenarios that differed in the distribution of connections from EN and pEN (deep layer) neurons (Fig. 5A, Top and Bottom). In scenario 1, each neuron in the EN and pEN generates more excitatory connections than each neuron in the piriform cortex, but those connections were distributed randomly across olfactory cortex, referred to as a power-law network (Fig. 5A, Top). In

Fig. 3. Network-wide burst similarity was partially reproduced in random but not columnar network models. (A) A color plot of the conductance over time for each neuron in a network simulation of 6,400 neurons with random (Upper) or columnar (Lower, 32 columns of 200 neurons) connectivity in response to simulated afferent (LOT) stimulation. (B) Example conductance traces from two cells on four trials of simulated stimulation of a network with random connectivity. The random network model partially recapitulated the intratrial burst shape similarity (compare black and red traces for each trial) yet intertrial variability (compare red or black traces across trials). (C) Example conductance traces from a columnar network model. Columnar models did not exhibit the intratrial burst waveform similarity that was observed in the experimental data. (D) Bar graph of the pairwise minus the within-cell CV ($\overline{CV}_{pair} - \overline{CV}_{cell}$) from recordings of olfactory cortex, random network models, and columnar network models.

Fig. 4. Network models with distributed subnetworks lacked the pairwise burst correlation seen in recordings of olfactory cortex. (A) The unsorted (Upper) and sorted (Lower) connectivity matrix for a network of 1,000 neurons with 10 subnetworks and 50% within-subnetwork connections. (B) Example set of four conductance traces from a network model with distributed subnetworks (80% within-subnetwork connections) did not reveal that pairwise intratrial burst similarity that was characteristic of the experimental results. (C) Bar graph of the pairwise minus the within-cell CV ($CV_{pair} - CV_{cell}$) for network models with distributed subnetworks and the experimental results.

scenario 2, connectivity was the same as in scenario 1, except that EN and pEN projections were biased toward other EN and pEN neurons, referred to as a hierarchical network (Fig. 5A, Bottom). An example of the traces generated by a hierarchical network model is shown in Fig. 5B, which exhibits intra- and intertrial burst-shape similarities similar to recordings (Fig. 1B). Network models with power-law or hierarchical topologies produced pairwise CV values substantially less than within-cell CV values, by an extent that spanned the range of experimentally observed values (Fig. 1C), suggesting that either topology could account for our experimentally observed results.

We therefore attempted to distinguish between simple power-law and hierarchical topologies experimentally. In the power-law network model, burst currents were the same across layers because the targets were random. In contrast, hierarchical network models had burst currents that were larger in the densely connected layer because neurons connected to each other with higher probability within that layer (ratio of peak amplitudes, 1.47 ± 0.14 ; $P < 0.05$). To determine which model was consistent

with the data we measured the magnitude of burst currents in AOC, APC_{v-r}, APC_{v-c}, and pEN. Consistent with the hierarchical model, burst currents were significantly larger in pEN than in other regions (Fig. 5C; $F = 61$). Supporting a driving role for pEN in the network response, action potential firing was most intense in pEN and the action potentials tended to occur first in pEN neurons (Fig. S4). Furthermore, when AOC was separated with a knife cut from the pEN and piriform cortex stimulation of AOC no longer generated bursts (Fig. S5), even though the AOC exhibits recurrent connectivity (22). Thus, the pairwise variance patterns seen in our recordings are most consistent with hierarchical connectivity critically involving the pEN.

Fig. 5. Hierarchical network models closely matched the burst patterns observed in recordings of olfactory cortex. (A) A diagram of a two-layer power-law excitatory network model (Upper) and hierarchical network model (Lower) motivated by the connectivity of the olfactory cortex. In both models, each deep-layer neuron generates more connections than each superficial-layer neuron. In the hierarchical model more of each deep-layer cell's connections are local. Horizontal green lines represent the incoming afferent fibers of the LOT. Blue cells represent piriform pyramidal neurons (superficial layer) and red cells represent the pEN and EN neurons (deep layer). Colored triangles represent presynaptic terminals and curved lines indicate their axons. Red dashed lines indicate connections that differ between the models. (B) Example conductance traces from a hierarchical network model. The hierarchical model exhibited intratrial burst waveform similarity observed in the experimental data (compare with Fig. 1B). (C) The pairwise minus the within-cell CV ($CV_{pair} - CV_{cell}$) from recordings of olfactory cortex were well captured by network models with power-law or hierarchical topologies. For power-law networks, gamma values varied from 3.25 (left bar) to 2.5 (right bar) in 0.25 increments. For hierarchical models, gamma values were 3.0 for the left two bars, and 2.5 for the right bars and the within network bias from left to right, was: [0.2, 0.3, 0.2, 0.4]. (D, Left) Example current traces of network bursts from representative cells in three subregions of olfactory cortex. (D, Right) Summary plot of the amplitude of network burst currents in each region. Network burst currents in pEN were substantially larger than those in other regions.

Discussion

We used pairwise variance analysis of short network-wide bursts of excitation to probe the topology of the excitatory network in olfactory cortex. Our results show that this network has the surprising ability to generate bursts of synaptic activity that are highly similar in time course across neurons throughout the cortex on a single trial, despite significant trial-to-trial variability in each neuron. Our analysis of burst-variance patterns in realistic large-scale network models demonstrated that power-law and hierarchical network models—but not columnar, random, or distributed subnetwork models—are capable of reproducing the recorded burst-variance pattern. Hierarchical models were most consistent with the data when subregion-specific timing and amplitudes of the bursts were taken into account.

Short Bursts of Excitation as a Probe of Network Topology. Transient widespread bursts, also called epileptiform bursts, are a ubiquitous feature of cortical structures ranging from the hippocampus (21), to neocortex (19), to the piriform cortex (20). They occur after *in vivo* kindling, with induction of synaptic plasticity, with disinhibition, or in normal conditions with paired stimulation (20, 30–32). The occurrence of network-wide bursts is closely related to the density of recurrent connections. The piriform cortex and CA3 region of the hippocampus are highly burst-prone, and probably not coincidentally, appear to have the densest associational connectivity in the brain (2, 3). In simulated network models, recurrent connectivity must have a sufficient density for transient bursts to occur (24). In addition, the strength of individual connections and background firing rates must be low (25, 26). These conditions match the properties of excitatory synapses and neuronal excitability in brain slices of olfactory cortex (22). However, isolated AOC did not generate network bursts in our experiments, so not all subregions of olfactory cortex are equivalent in this regard.

We observed that the fine structure of network bursts in olfactory cortex exhibit a surprisingly similar time course across neurons in the network, despite significant trial-to-trial variability. Unlike the bursts themselves, which are ubiquitous to cortical circuits, the pairwise variance structure of the bursts was reproducible in hierarchical and power-law network models, but not other models, thus providing means to discriminate between network topologies. In particular, the variance structure in our data were markedly different from that observed in models with prominent subnetwork architecture, whether distributed (33) or local (1). The distinctive network structure in olfactory cortex is supported by the short lags between bursts in different subregions and the known distributed connectivity (2, 3, 15). Blocking NMDA receptors did not significantly alter the shape of the early component of the bursts, supporting that the trial-to-trial variability of the bursts was a robust indicator of network topology rather than an artifact of the particular level of excitation.

It has been suggested that connectivity in the olfactory cortex appears random (8). Simulated random networks support widespread coherent activity, as well as associative memory (34), a proposed function for piriform cortex (2, 3). However, random networks were poorly predictive of the pairwise variance patterns in our data. Furthermore, the tendency of the deep subregions of the piriform network to drive activity suggests a nonrandom organizational feature such as that of a power-law or hierarchical network, as supported by our measurements of burst magnitude and timing. Because of their enhanced connectivity with other regions, pEN and EN neurons may act as network hubs (15). Furthermore, dense connectivity within pEN and EN suggests a second level of hub architecture, a hallmark of power-law, scale-free networks, or hierarchical networks that can support

widespread coherence (35, 36) and confer computational advantages (25, 36).

Inhibition and the Structure of the Excitatory Network. Although our experiments were conducted under disinhibited conditions to allow us to isolate the excitatory network, normal cortical activity of course is shaped by inhibition (30). However, block of inhibition has been used to good advantage in other cortical areas to focus on relevant network dynamics, revealing spiral waves in neocortex (37) and laminar features of excitatory circuits in motor cortex (38). The variance patterns of burst activity in our experiments, when combined with network modeling, provided insight into the architecture of the intrinsic excitatory circuitry as well as the functional excitatory connectome on the synaptic scale. The question remains, how does the hierarchical topology in olfactory cortex shape activity *in vivo* and with inhibition intact? One way to view this question is to think of a network as based on a foundation layer of excitatory connectivity that then is further shaped by superimposed inhibitory and neuromodulatory inputs. According to this view, inhibition may raise and lower the overall threshold across large regions on the timescale of the synaptic activity we observe, and thus primarily serve as a gain control mechanism (39). Alternatively, it is possible that inhibition shifts the dynamics of the circuit, in which case the role of hierarchical excitatory connectivity may be masked or transformed. Unfortunately, we were not able to use our analytical approach with inhibition intact because network bursts did not occur after single weak LOT shocks, presumably because of simultaneous and synchronous activation of large numbers of GABAergic interneurons. However, it is important to remember that under natural conditions afferent activation of subsets of olfactory cortical principal neurons is not nearly as synchronous as a single shock to the LOT (40). Further experiments, perhaps using naturalistic spatiotemporal stimulation, will be necessary to determine how inhibition interacts with the hierarchical excitatory connectivity in the olfactory cortex.

Implications for Olfactory Cortical Processing. The basic coding strategy in olfactory cortex remains an enigma. In many sensory systems, the cohorts of cortical neurons that process a particular feature of sensory space show modular clustering (1). The widespread shape similarity of network bursts in olfactory cortex that we observed suggests a distributed excitatory network. This may help support high-frequency interactions over long distances. The olfactory cortex is driven by the olfactory bulb at gamma frequencies (40–100 Hz), particularly in connection with learning (41–44). Prominent oscillatory activity involves the piriform cortex at beta frequencies (15–35 Hz), also associated with learning (42, 44, 45). Structured activity in endopiriform nucleus in behaving rats can reach frequencies up to 200 Hz (46). Given the distributed nature of connectivity in olfactory cortex (2–4), it is apparently capable of supporting widely distributed coherent ensemble activity over a wide range of frequencies. We hypothesize that this capability is supported by the boosting effect of hierarchical connectivity with the endopiriform and preendopiriform nuclei. We expect that with inhibition intact, coherence will be temporally restricted to smaller, although still distributed, ensembles, providing a substrate for fast distributed population coding (47). Fast interactions in distributed and overlapping cortical ensembles may be essential to accomplish a hierarchical coding of the high-dimensional odor space (48).

Materials and Methods

Brain-Slice Preparation and Electrophysiological Recording. Slices of the olfactory cortex (300 μ m, Leica VT 1200S) were prepared from C57BL/6J mice at age P21–P28. Excitatory connectivity in rodent olfactory cortex is well established at this age (49). Standard procedures approved by the institutional IACUC were used for slice preparation (*SI Materials and Methods*). Neurons in brain slices were visualized using IR-differential interference

contrast (DIC) optics through a 40 $\times$ objective on a Zeiss Axioskop microscope. Principal neurons were targeted using morphological and physiological criterion (*SI Materials and Methods*). Whole-cell patch-clamp recording and electrical stimulation were conducted using standard approaches (*SI Materials and Methods*). The stimulating electrode was placed in the LOT above the AOC.

Network Modeling and Statistics. Cortical network models were constructed in Matlab (The Mathworks). All models consisted of 6,400 neurons organized in a line representing the long, approximately rostrocaudal axis of the piriform cortex. Neurons were connected with an average probability of 0.2%. A fixed synaptic delay was added for each synaptic connection based on the distance between them such that total delay from end to end was 5 ms. Models with local (columnar) or distributed subnetworks had 32 subnetworks with 200 neurons each. Networks with power-law or hierarchical structure had a densely connected layer consisting of 320 neurons evenly spaced along the line network that did not receive direct afferent stimulation (referred to as the deep or hidden layer).

Intrinsic membrane properties were simulated using a system of two differential equations with a reset condition, following Izhikevich (50) (*SI Materials and Methods*). To model stimulation of the afferent pathway (the LOT), 10% of the neurons in the input layer were synchronously activated with an alpha synapse. The mean strength of recurrent synapses was adjusted for each network model to be just above the threshold necessary to result in transient bursts on repeated trials of simulated stimulation. Each recurrent synaptic connection, C_{ij} , was endowed with its own synaptic strength, short-term plasticity dynamics, and variability using a modification of an algebraic solution to the simple model by ref. 51, with parameters adjusted to match recordings of piriform cortex intracortical synapses (22) (*SI Materials and Methods*).

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