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Correlated firing in tufted cells of mouse olfactory bulb

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Abstract

Temporally correlated spike discharges are proposed to be important for the coding of olfactory stimuli. In the olfactory bulb, correlated spiking is known in two classes of output neurons, the mitral cells and external tufted cells. We studied a third major class of bulb output neurons, the middle tufted cells, analyzing their bursting and spike timing correlations, and their relation to mitral cells. Using patch-clamp and fluorescent tracing, we recorded spontaneous spiking from tufted-tufted or mitral-tufted cell pairs with visualized dendritic projections in mouse olfactory bulb slices. We found peaks in spike cross-correlograms indicating correlated activity on both fast (peak width 1 ms – 50 ms) and slow (peak width > 50 ms) time scales, only in pairs with convergent glomerular projections. Coupling appeared tighter in tufted-tufted pairs, which showed correlated firing patterns and smaller mean width and lag of narrow peaks. Some narrow peaks resolved into 2–3 sub-peaks (width 1–12 ms), indicating multiple modes of fast correlation. Slow correlations were related to bursting activity, while fast correlations were independent of slow correlations, occurring in both bursting and non-bursting cells. The AMPA receptor antagonist NBQX (20 μ M) failed to abolish broad or narrow peaks in either tufted-tufted or mitral-tufted pairs, and changes of peak height and width in NBQX were not significantly different from spontaneous drift. Thus, AMPA-receptors are not required for fast and slow spike correlations. Electrical coupling was observed in all convergent tufted-tufted and mitral-tufted pairs tested, suggesting a potential role for gap junctions in concerted firing. Glomerulus-specific correlation of spiking offers a useful mechanism for binding the output signals of diverse neurons processing and transmitting different sensory information encoded by common olfactory receptors.

Keywords

cross-correlogram; glomerulus; action potential; bursting; mitral cell; gap junction

The mammalian olfactory bulb contains hundreds or thousands of glomeruli, each of which receives convergent fibers of sensory neurons expressing a unique olfactory receptor chosen from a large family of G-protein coupled receptors (Buck and Axel, 1991; Mombaerts et al., 1996; Firestein, 2001). Different olfactory receptors are tuned to recognize different odorants, and hence odors are encoded at the input stage by specific combinations of activated glomeruli (Malnic et al., 1999; Johnson and Leon, 2007; Wachowiak and Cohen, 2001; Uchida et al., 2000). Each glomerulus incorporates a modular circuit composed of output neurons (mitral and tufted cells) and local interneurons (periglomerular cells) interacting through an intricate dendrodendritic network (Pinching and Powell, 1971;

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Kosaka et al., 2005; Chen and Shepherd, 2005). The glomerular circuit is activated when an odorant stimulus triggers release of glutamate from sensory neuron terminals, leading to the excitation of primary (apical) dendrites of mitral and tufted cells which relay their spike output directly to olfactory cortical targets (Murphy et al., 2004; Wachowiak and Shipley, 2006; Aroniadou-Anderjaska et al., 1997).

A fundamental problem in olfactory coding is to determine the input-output transformation of the olfactory bulb neural network. What is the relationship between mitral and tufted cell outputs and olfactory receptor-coded inputs received by the glomerular array? Synaptic inhibition from periglomerular cells and granule cells could filter the input and reshape the spiking of output neurons, potentially restructuring the combinatorial glomerular code. For example, feed-forward and feedback inhibition acting in a glomerular circuit could implement thresholding or impose temporal structure on principal neuron spike trains (Jahr and Nicoll, 1982; Gire and Schoppa, 2009), and lateral inhibition could enhance contrast between output signals of different glomeruli (Mori et al., 1999; Yokoi et al., 1995; Urban and Sakmann, 2002). Such processing functions are often assumed to operate on glomeruli as irreducible units representing specific molecular features encoded by olfactory receptors. However, an additional layer of complexity arises from the divergent relay of glomerular output to multiple mitral and tufted cells. Studies on rodents have counted an average of 10–20 mitral cells per glomerulus (Royet et al., 1998), and at least twice as many tufted cells (Meisami and Safari, 1981). Do all output cells from one glomerulus send identical messages to downstream neurons? There is evidence that homotypic output neurons may be specialized to process different kinds of odor information. Compared to tufted cells, mitral cells have broader dendritic fields, are located deeper in the bulb, and probably engage different granule cell populations (Orona et al., 1983; Orona et al., 1984; Mori et al., 1983). They also differ in their cortical projections: mitral cells innervate the rostrocaudal extent of olfactory cortex, while tufted cells project to more rostromedial areas (Haberly and Price, 1977; Schoenfeld and Macrides, 1984; Ekstrand et al., 2001; Schneider and Scott, 1983; Scott, 1981). Mitral and tufted cells show differing spike responses to electrical stimulation (Ezeh et al., 1993; Schneider and Scott, 1983) and odorants (Nagayama et al., 2004; Griff et al., 2008). A recent survey of odorant responses of mitral/tufted cells receiving input from the I7 olfactory receptor revealed variation in tuning at higher stimulus concentrations (Tan et al., 2010). Specialization of output neurons may also occur in the antennal lobe, the insect analog of the olfactory bulb. In the macroglomerular complex of the moth, projection neurons from the same glomerulus can exhibit different spiking responses to odorants (Christensen et al., 2000).

Functional heterogeneity of homotypic output neurons does not necessarily mean that the receptor-specific glomerular code is scrambled. Integrity of the code could be preserved by a temporal binding mechanism involving spike correlation or synchronization. For example, projection neuron spiking in the moth shows significantly stronger correlation (at spike lags < 5 ms) in cell pairs linked to the same glomerulus, than in pairs linked to neighboring glomeruli (Lei et al., 2002). Similar fast correlations in spiking (< 5 ms) occur in homotypic projection neurons of *Drosophila* antennal lobe receiving common olfactory receptor input (Kazama and Wilson, 2009). In the rodent olfactory bulb, there are precise temporal correlations of spikes (within a few ms) in homotypic mitral cells (Schoppa and Westbrook, 2002). On slower time scales, long-lasting depolarizations of several hundred ms also show glomerulus-specific correlation (Christie et al., 2005; Carlson et al., 2000; Schoppa and Westbrook, 2001). In olfactory bulb of *Xenopus*, mitral/tufted cells linked to the same glomerulus exhibit slow synchronous calcium transients, and spike timing is correlated to within 10 ms (Chen et al., 2009).

If temporally correlated spiking is the ‘glue’ that binds homoglomerular output into coherent signals, then we would expect spike correlations in all pairs of output neurons linked to the same glomerulus. The tufted cells comprise a major class of mammalian olfactory bulb output neurons whose spike correlations have not been studied in detail. Considering the possibly distinct roles played by mitral and tufted cells in olfactory coding, we asked whether spike output of tufted cells was temporally correlated with that of mitral cells of the same glomerulus. We recorded spike activity in middle tufted cells of mouse olfactory bulb slices, using a fluorescent dye to trace apical dendrites into the glomerular layer. We found both slow and fast glomerulus-specific correlations in spontaneous spiking among tufted cells, and between mitral and tufted cells.

EXPERIMENTAL PROCEDURES

Slice preparation

Slicing and recording procedures were similar to those employed in previous studies (Ma and Lowe, 2004; Ma and Lowe, 2007). Olfactory bulb slices were prepared from male mice (P21-28, CD-1 strain, Charles River Laboratories, Wilmington, Massachusetts). Animals were sacrificed by decapitation under deep halothane anesthesia, the bulbs extracted and transferred immediately to ice cold sucrose slicing solution containing (mM): 240 sucrose, 2.5 KCl, 10 Na-HEPES, 10 glucose, 1 CaCl₂, 4 MgCl₂, 0.2 ascorbic acid, pH 7.3. Horizontal slices (300–400 μ m) were cut and incubated (1–3 h, 32°C–23°C) in an interface chamber containing (mM): 124 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 10 glucose, 1 CaCl₂, 3 MgCl₂ (95% O₂, 5% CO₂). During dye loading and patch-clamp recording, slices were submerged and immobilized with a platinum harp and perfused (2 ml/min) with ACSF (mM): 124 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 25 glucose, 2 CaCl₂, 50 μ M MgCl₂ (95% O₂, 5% CO₂, 23°C). Low extracellular Mg²⁺ (50 μ M) was used to boost basal firing activity of principal neurons. Cells in slices were visualized with differential interference contrast optics on an Olympus BX50-WI microscope equipped with a 60 $\times$ NA 0.90 water immersion objective (Olympus America Inc., Center Valley, PA, USA), and viewed on a video monitor. Extracellularly applied drugs were delivered by switching perfusion reservoirs under open flow mode, or by injection into the flow line under recycled (closed loop) flow mode. ($\pm$)-4-(4-aminophenyl)-2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) disodium was obtained from Sigma-Aldrich (St. Louis, MO, USA). For glutamate uncaging controls, we used 4-methoxy-7-nitroindolyl (MNI-) glutamate (Tocris, Bioscience, Ellisville, MO, USA), photolyzed by a xenon flash lamp (Cairn Research Ltd., Faversham, UK). Unless otherwise stated, all other chemicals were obtained from Sigma-Aldrich.

All experimental procedures adhered strictly to guidelines for animal care, handling and euthanasia set by the U.S. Public Health Service. All protocols were reviewed and approved by an Institutional Animal Care and Use Committee.

Cell tracing

Fluorescent tracing was applied to visualize projections of primary dendrites of mitral and tufted cells in slices prior to patch-clamp recording. Somata of mitral and tufted cells were located by Nomarski imaging and randomly selected cells were loaded with tetramethylrhodamine dextran (3,000 MW) (Invitrogen Corp., Carlsbad, California) by single-cell electroporation (Olofsson et al., 2003; Rae and Levis, 2002). Tips of patch pipettes (8–10 M Ω) filled with dye solution (1–3 mM) were pressed against the somatic membrane (loose patch, < 20 M Ω seal) and dye was ejected by a train of voltage pulses (1 ms, 100 Hz, 500 – 700 ms duration, –5 to –8 V) delivered by an Axoparator 800A (Molecular Devices, Sunnyvale, California). Simultaneous cell-attached recordings showed

that loading by electroporation sometimes caused a temporary drop in spike rate, but normal activity was recovered 30 – 60 min afterwards. Slices containing loaded cells were incubated for 60 min in the interface chamber to allow recovery from electroporation and retrograde dye filling of dendrites. For fluorescence excitation, we used light from a 100 W mercury short arc (HBO103 W/2, OSRAM, Augsburg, Germany) passed through an interference filter set for tetramethylrhodamine (excitation 525 nm, dichroic 560 nm, emission 565; XF101-2, Omega Optical, Brattleboro, Vermont). Fluorescently labeled dendrites were visualized with a low-light CCD video camera (V-1070, Marshall Electronics, El Segundo, California) and images of cell morphology acquired by digitizing the video signal with a frame grabber board (PCI-1407, National Instruments, Texas).

Electrophysiology

Patch-clamp recordings of spontaneous spiking in cell pairs were made in tight seal cell-attached voltage clamp, or whole-cell voltage clamp or fast current clamp modes, using Axopatch-1C and Axopatch-1D amplifiers (Axon Instruments, Burlingame, California). Amplifiers were controlled through ITC-18 A/D-converters (Instrutech corporation, New York) by either a single computer running WinEDR 3.0.3 (University of Strathclyde, UK) or a pair of computers running Pulse 8.5 software (HEKA Elektronik, Lambrecht, Germany). Signals were filtered (4-pole low pass Bessel, –3 dB roll-off at 1 – 2 kHz) and digitized at a sample interval of 100 – 300 μ s. For experiments controlled by two computers, we routed the second amplifier output to an additional non-multiplexed channel on the first computer interface to calibrate the timing of acquisition. Accurate recording of signals from the two amplifiers was verified by dual patch-clamp recordings of action potentials from the same neuron. Due to variable recording conditions, patch electrodes recorded either spike capacitance currents under cell-attached voltage clamp, or action potentials under whole-cell current clamp. We verified that in the minority of cases in which two electrodes recorded under different modes, a time shift of < 1 ms was introduced into the spike peak positions (corresponding to approximately half the upstroke of a single action potential). We did not apply any correction for this in spike correlation analyses because we used time bins of 1 ms or longer. To facilitate dual recordings from labeled cells in different locations of the slice, we used computer-controlled microscope positioning. The microscope optics were mounted on an XY platform driven by DC motors and a controller board (M227.25, C-842; Polytec PI, Auburn, Massachusetts) commanded by custom software written in LabVIEW (National Instruments, Austin, Texas). Patch-clamp recording pipettes (6 – 10 M Ω) were filled with potassium methylsulfate solution, containing (mM): 122 K-CH₃SO₄, 5 KCl, 26 K-HEPES, 2 Mg-ATP, 0.3 Na-GTP, 1 MgCl₂, 4 Na₂-phosphocreatine, 6.5 biocytin HCl (pH 7.2), supplemented with 200 μ M Alexa Fluor 594. If the whole-cell configuration was obtained, fluorescent imaging was maintained by diffusion of Alexa Fluor 594 into the cell (with filters: excitation 560 nm, dichroic 590 nm, emission 610 (XF40-2, Omega Optical)). After recording, slices were fixed overnight (4°C) in phosphate-buffered saline with 2% glutaraldehyde. Biocytin filled cells were stained using the Vectastain Elite ABC kit and VIP peroxidase substrate kit (Vector Laboratories, Burlingame, California), and examined as whole mounts in 80% glycerol with a Nikon DXM1200C digital camera attached to a Nikon Eclipse 80i microscope (Nikon Inc., Melville, NY, USA).

Data analysis

Paired current or voltage records of long duration (38 – 200 s) were acquired using WinEDR 3.0.3. Local maxima in these records corresponding to action potential peaks were detected using Mini Analysis 6.0.3 (Synaptosoft, Inc., Decatur, Georgia). Spiking patterns were characterized by plotting histograms of interspike interval (ISI) distributions, and calculating the mean spike rate (= number of spikes in entire record/duration of entire record) and coefficient of variation (CV= ISI standard deviation/ISI mean). A cell was classified as

bursting if the log-linear ISI histogram displayed a peak on the left with a discontinuity of > 30% in either slope or height in its decay with increasing ISI (e.g. Fig. 1B, arrow on inset). This empirical criterion was useful for capturing the global tendency of the entire spike train to have abrupt transitions in firing rate that accompanied entry or exit from a burst. A burst was operationally defined as a series of 3 or more consecutive spikes with ISI less than a predetermined threshold value, corresponding to discontinuity in the log-linear ISI histogram. This method allowed us to recognize overall bursting patterns of activity, independent of intraburst (IB) frequency which varied widely across the cell population. Bursting patterns were visualized in joint ISI histograms by plotting consecutive ISI values of spikes (e.g. Fig. 1C) (Reinagel et al., 1999; Rodieck et al., 1962; Zeck and Masland, 2007). Custom LabVIEW software was written to detect and analyze bursts, and compute the following parameters: burst fraction (= number of spikes in bursts/total number of spikes), mean burst rate (= number of bursts in entire record/duration of entire record), interburst CV (= standard deviation of interburst interval/mean interburst interval, with intervals taken between first spike in a burst), mean spikes per burst, mean burst duration (= time between first and last spike in a burst), burst duration CV (= standard deviation of burst duration/mean burst duration), mean intraburst ISI, mean intraburst spike rate (= 'mean burst spike rate' = mean value of all the reciprocal of intraburst ISIs), and intraburst CV (= ISI standard deviation of burst spikes/ISI mean of burst spikes).

The spike cross-correlogram was computed by using another LabVIEW program to histogram the spike lags between the two series of spike times, either with 10 ms bins to detect slower correlations, or 1 ms bins to detect faster correlations. Bin counts were normalized relative to the expected number of spikes per bin for pairs of neurons generating two independent Poisson-distributed spike trains with the same mean firing rates (Mann-Metzer and Yarom, 2000; Abeles, 1982). In some cases we averaged cross-correlograms computed from several acquired long records of the same duration to reduce noise. To test for significant peaks in the correlogram, we used a threshold of three standard deviations (99% confidence) above the normalized baseline ($1 + 3 \times \text{SD}$), where SD (standard deviation) was either: (i) calculated at zero lag for the two Poisson spike trains, or (ii) estimated from bin count fluctuations over a central lag segment (± 200 ms) of the shuffled cross-correlogram obtained by shifting spike times of the two cells by half a record length (Perkel et al., 1967). The shuffle-estimated SD tended to be smaller than the Poisson value by $8.6 \pm 16.5\%$ ($n = 56$). Both SD criteria identified the same set of cross-correlogram peaks. Peak shapes were variable, depending on firing properties of individual cells. Some peaks in normalized cross-correlograms were flanked by dips below unity that would be expected from spike clustering, but we did not attempt to model these phenomena. For standardized comparisons, we simply described peaks by three parameters (Fig. 5D, right inset panel), height (H) above normalized baseline, width at half height (W) and lag (L), derived by using the Levenberg-Marquardt nonlinear least squares algorithm to fit a Lorentzian function with unity offset:

$$y = 1 + H \frac{W^2}{4(x - L)^2 + W^2}$$

If narrow peaks were superimposed upon broad peaks, we deleted the narrow peak to obtain a fit to the broad peak alone (e.g. Fig. 5A), and subtracted the broad fitted curve to isolate the narrow peak for fitting to a Lorentzian with zero offset. Double narrow peaks were fitted by adding a second Lorentzian term to the formula. Variation in record lengths (38 – 200 s) did not strongly affect our estimation of these parameters. We compared peaks in the cross-correlogram of a 200 s data record with peaks in the averaged cross-correlograms of ten records of length 20 s obtained by splitting the original record. Values of H and W differed

by 5% or less, and L was shifted by less than 1 ms. In earlier experiments performed using Pulse 8.5 software, spike records were acquired episodically in shorter (2 s) records (Mann-Metzer and Yarom, 2000). To analyze and integrate these with our other data, we concatenated traces into long records, joined by gaps of 260 ms (measured hardware delay between consecutive sweeps) and applied Monte Carlo simulation to linearly interpolate spike firing rate across gaps (Poisson process, 1.2 ms pulses to simulate spikes, with 5 ms refractory period). To estimate the error in this method, we compared peak parameters of continuous records from 5 pairs with those obtained by simulated episodic sampling and concatenating the same records. We found that H and W values varied by < 15 % (9/10 H or W deviations < 10%), and L was shifted by < 5 % of W . The set of peaks flagged as significant by the $3 \times \text{SD}$ criteria did not change.

In addition to peak descriptors H , W and L , we also calculated two other metrics (Fig. 5E, inset) to quantify central peak tendency of cross-correlograms, that could be used to compare correlograms that fell below the $3 \times \text{SD}$ criterion. Using 10 ms binned correlograms, we defined: (i) the central peak height, ch , as the difference between the mean bin values over $t_{\text{max}} \pm 10$ ms, and over flanking intervals out to $t_{\text{max}} \pm 100$ ms, where t_{max} is the lag time corresponding maximum amplitude within the central interval $[-100 \text{ ms}, 100 \text{ ms}]$, and (ii) the side area, sa , as the integrated area of the correlogram, $C(t)$, within the interval $t_{\text{max}} \pm 150$ ms, excluding the interval $t_{\text{max}} \pm 30$ ms:

$$sa = \int_{t_{\text{max}}-150 \text{ ms}}^{t_{\text{max}}-30 \text{ ms}} C(t) dt + \int_{t_{\text{max}}+30 \text{ ms}}^{t_{\text{max}}+150 \text{ ms}} C(t) dt$$

The value of ch is a measure of the strength of fast correlation (< 10 ms), subtracting out slower correlations (10 ms – 100 ms), and for correlograms with significant fitted peaks, ch was well correlated (Pearson's $r = 0.87$, $n = 36$) with the heights of narrow peaks ($W < 60$ ms). The value of sa is a measure of slower correlations, excluding faster correlation (< 30 ms), and for significant fitted peaks it was well correlated (Pearson's $r = 0.98$, $n = 31$) with the heights of broad peaks ($W > 50$ ms). For standard errors of ch values, we took the shuffled correlogram standard deviation ($\text{SD}_{\text{shuffle}}$) as an estimator of correlogram bin error, giving $\text{SD}_{ch} = \sqrt{(\sigma_{10}^2 + \sigma_{90}^2)}$, where the variances are $\sigma_{10, 90} = \text{SD}_{\text{shuffle}}/\sqrt{N_{10, 90}}$, and $N_{10, 90}$ the number of bins for each mean value (central 10 ms and two flanking 90 ms long intervals). For standard errors of sa values, we computed the side areas of the shuffled cross-correlograms.

For general data analysis, we used Origin 7.0 software (OriginLab Corp., Massachusetts) for nonlinear least squares fitting and summary statistics. Results are expressed as mean $\pm$ SD, unless otherwise stated. P values of confidence limits on differences in sample distributions were obtained from Students t-test or Mann-Whitney U-test (comparing means), and Kolmogorov-Smirnov (KS) test (comparing means and distributions), using Statistica 8.0 (StatSoft, Tulsa, Oklahoma). Some P values were obtained by direct calculation from the binomial distribution. Correlation in scatter plots was assessed by least square linear regression, citing the value of Pearson's correlation coefficient (R value).

RESULTS

Spontaneous firing patterns of mitral and tufted cells

To establish a baseline for the analysis and comparison of spike correlations in tufted cells and mitral cells, we first characterized the spontaneous spiking patterns of cells in olfactory bulb slices. Tufted cells may include several distinct subtypes. For example, superficial tufted cells that reside just below the glomerular layer are distinguished by intrabulbar projections and expression of cholecystokinin (Liu and Shipley, 1994; Kosaka and Kosaka,

2007; Seroogy et al., 1985), while inner tufted cells (displaced mitral cells or Type II mitral cells) have extensive, symmetric secondary dendritic fields similar to mitral cells (Orona et al., 1984; Macrides and Schneider, 1982; Mori et al., 1983). To acquire data from a more homogenous population, we specifically targeted the middle tufted cells positioned at intermediate levels of the external plexiform layer (EPL). Compared to mitral and inner tufted cells, these have less extensive, more asymmetric secondary dendrites (Orona et al., 1984; Macrides and Schneider, 1982). Cells were loaded by electroporation with fluorescent dye, and their identity as tufted cells visually confirmed by tracing primary dendrites into the glomerular layer (Fig. 4A). In recordings from 83 spontaneously firing tufted cells, we observed wide variation in spiking patterns, ranging from strongly bursting to non-bursting (Figs. 1A, 1F). Bursting cells typically exhibited a bimodal ISI histogram, the left peak corresponding to intraburst ISIs, the right peak to interburst ISIs (Fig. 1B). In the joint ISI histogram, the spikes were distributed in an L-shaped pattern that resolved into 4 quadrants: solitary spikes (upper right), burst or doublet initiators (lower right), burst or doublet terminators (upper left), and intraburst spikes (lower left). Bursting was usually irregular, with high variability of the interburst interval (IBI). For example, the IBI distribution of the bursting cell shown in Fig. 1A was nearly Poisson, with interburst CV = 0.85 (Fig. 1D). The intraburst spike rates typically varied by roughly 2-fold for a given cell, but mean intraburst spike rates varied widely, by more than 10-fold over the entire population (13 – 164 Hz). An example of a non-bursting tufted cell is shown in Figs. 1F–1H. The ISI distribution was unimodal and Poisson distributed (CV = 1.02), and the distribution of spikes in the joint ISI histogram was dispersed and unstructured. In our sample of tufted cells, there were 34 non-bursters and 49 bursters. Although the fraction of bursters was slightly higher (59%) than for mitral cells, the number was not significantly greater than expected from random chance ($P = 0.06$).

We performed the same analyses on spike records obtained from 41 mitral cells and again found wide variation in spiking patterns, including bursting and non-bursting cells. Fig. 2A shows a record from a bursting mitral cell, which had a bimodal ISI distribution (Fig. 2B), L-shaped spike distribution in the joint ISI histogram (Fig. 2C), and irregular IBIs (Fig. 2D). In contrast, the non-bursting mitral cell shown in Fig. 2F–2H had a unimodal, nearly Poisson ISI distribution (CV = 0.90), and an unstructured joint ISI histogram. In our sample of mitral cells, there were 21 non-bursters and 20 (49%) bursters. This fraction of bursters was not significantly greater than expected from chance ($P = 0.5$).

Parameters characterizing spiking and bursting in both mitral and tufted cells are organized and summarized in Fig. 3 and Table I. Previous studies showed that tufted cells responded more robustly to odorant stimuli *in vivo*, and they may be under weaker inhibitory control (Griff et al., 2008; Nagayama et al., 2004). We expected to see this reflected in the spike activity in slices. However, our comparisons did not reveal any significant differences between the distributions of basal spiking and bursting parameters of the two cell types (KS tests, $P > 0.10$). Differences in inhibitory control of homotypic principal neurons might not be easily detected in a basal state of low spontaneous activity, and may only be revealed under strong external stimulation (Tan et al., 2010).

Correlated firing in tufted–tufted cell pairs

We expected correlations in spike firing to be related to glomerular projections, so we sorted mitral and tufted cell pairs into two categories based on fluorescent imaging of apical dendrites: those with apical tufts that were visibly overlapped in a focal plane were classified as *convergent*, and those with non-overlapping tufts were classified as *divergent*. To minimize experimental perturbation of spike firing properties of cells in glomerular networks, we mostly recorded non-invasively in cell-attached mode, seeking spontaneously active pairs that did not require external current injection to evoke spiking. In a minority of

cases, we subsequently obtained stable whole-cell access to both cells and used biocytin filling and staining to confirm tuft overlap (Fig. 4B). Due to a low success rate for achieving double fills after prolonged cell-attached recording, we could not routinely stain the tufts of both cells, so we cannot exclude that some convergent primary dendrites may have projected to closely neighboring glomeruli, rather than the same glomerulus.

We recorded from 18 spontaneously spiking pairs of middle tufted cells with convergent primary dendrites, and in 17/18 pairs detected at least one significant peak in the cross-correlogram. We found correlograms with peaks in three categories (Figs. 4D, 4F, 4H): Fig. 4C shows a record from a strongly bursting pair with a broad cross-correlogram peak (Fig. 4D), indicating strong correlation on slow time scales (> 100 ms); Fig. 4E shows a record from another bursting pair with both a broad and a narrow peak superimposed (Fig. 4F), indicating correlation on both slow (> 100 ms) and fast (< 10 ms) timescales; Fig. 4G shows a record from a weakly bursting pair, with the correlogram dominated by a large narrow peak (Fig. 4H), indicating strong correlation only on faster time scales (< 10 ms). The fraction of pairs in each category was: broad peak only 3/17 (17.6 %), both broad and narrow peaks 10/17 (58.8%), and narrow peak only 4/17 (23.6 %). The occurrence of narrow peaks did not depend on the presence or absence of broad peaks. Narrow peaks were observed in 4/5 pairs without broad peaks, and 10/13 pairs with broad peaks, rates not significantly different from those expected from an overall narrow peak fraction of 14/18 = 78% ($P = 0.30$ and 0.32 respectively). We also noted an absence of correlation between narrow peak height and side area for cells with convergent dendrites ($R = 0.03$, $P = 0.92$, $n = 16$) (Fig. 5G). This independence suggests that different mechanisms are responsible for slow and fast correlations.

In 5 tufted-tufted cell pairs, we were able to resolve fine structures of narrow peaks in cross-correlograms using 1 ms bins. Fig. 5A shows the 10 ms binned correlogram of a cell pair with a narrow peak and broad peak, the latter fitted to a Lorentzian curve. In Fig. 5B, the broad fitted curve was subtracted from the correlogram to isolate the narrow peak, shown with 1 ms bin width. This narrow peak resolved into sub-peaks, including two moderately narrow peaks with positive and negative lags ($L = -3.7$ ms, $+5.6$ ms, widths $W = 4.1$ ms, 6.9 ms respectively), and a sharp center peak in a 1 ms bin. The sub-peaks correspond to three modes of correlated firing at faster time scales, two moderate precision modes in which either member of the tufted-tufted pair fired a spike several ms before the other, and a high precision mode in which both cells fired within 1 ms of each other. A sharp center peak ($W < 2$ ms) was detected in 4 of the 5 pairs, occurring together with one or more moderately narrow ($W > 4$ ms) off-center peaks. Another example, with sharp and moderately narrow peaks, is shown in Fig. 5C, from a pair without a broad peak, showing that splitting of peaks in fast correlations did not require slow correlations to be present. A logarithmic plot of lags and widths for the set of fitted correlogram peaks from all tufted-tufted cell pairs, including resolved sub-peaks, revealed a gap in the distribution of widths (Fig. 5D). Thus, the peaks could be sorted into two groups based on peak width: narrow peaks ($1 \text{ ms} < W < 50 \text{ ms}$) and broad peaks ($50 \text{ ms} < W < 1 \text{ s}$). This dichotomy confirms that tufted cells with convergent dendrites engage in at least two distinct modes of correlated firing, i.e. a slow and a fast mode.

The occurrence of correlated firing was dependent on the convergence of apical dendrites. Of the 18 pairs with convergent dendrites, a broad peak was present in 13 pairs (72%), significantly greater than chance ($P = 0.048$); a narrow peak was present in 14 pairs (78%), also greater than chance ($P = 0.015$). In contrast, 0/8 pairs with divergent dendrites had any peaks, much less than chance occurrence ($P = 0.008$). To further evaluate the relation between correlation and dendritic convergence, we measured center height and side area of cross-correlograms for all pairs. For pairs with divergent dendrites, side area was not significantly different from zero ($P = 0.38$, $n = 8$, t-test), whereas for pairs with convergent

dendrites and broad peaks, it was significantly greater than zero ($P = 0.01$, $n = 13$) (Fig. 5F). Center height was also significantly greater for pairs with convergent dendrites ($n = 14$) compared to those with divergent dendrites ($P = 0.0001$, $n = 8$, Mann-Whitney test) (Fig. 5E). As another test of glomerulus-specific correlation, we compared maximum values of cross-correlograms in the interval ± 100 ms around zero spike lag, to the $1 + 3 \times \text{SD}$ criterion thresholds based on standard deviations of correlogram bin fluctuations (see Methods). The maximum value was not significantly greater than $1 + 3 \times \text{SD}$ for the set of pairs with divergent dendrites (paired t-test, $p = 0.925$ for SD from shuffled cross correlogram, 0.982 for SD from Poisson distribution, $n = 8$), but was greater for pairs with convergent dendrites ($p = 0.00019$ for shuffled SD, 0.00022 for Poisson SD, $n = 18$). Only one out of 18 pairs with convergent dendrites had its maximum value below $1 + 3 \times \text{SD}$. Thus, slow and fast correlated firing occurred when apical dendritic tufts were in close proximity or overlapping, and correlations most likely emerge through intraglomerular or nearby interglomerular couplings between the cells.

If correlated spiking of tufted cells arises from common glomerular pathways, we might expect cell pairs with convergent dendrites to express similar firing patterns. Indeed, we found that spike CV (which measures irregularity of firing), burst fraction (which measures degree of bursting) and mean burst rate, were positively correlated across pairs with convergent dendrites (Figs. 5H, 5I, 5J). The presence or absence of bursting was tightly linked among the convergent pairs: neither cell was bursting in 6/18 pairs, both were bursting in 10/18 pairs, but only 2 pairs had just one member of the pair bursting. This low number of single burster pairs deviated significantly from the expected frequency (50%) for random, independent bursting ($P = 7 \times 10^{-4}$). For divergent pairs, there was no significant deviation from frequencies expected for independent occurrence of bursting: 6/8 divergent pairs were single burster pairs, which was at chance level ($P = 0.15$).

Correlated firing in mitral–tufted cell pairs

The presence of correlated firing among tufted cells linked to the same glomerulus has a parallel in mitral cells, which also express glomerulus-specific spike correlations on both slow and fast time scales (Schoppa and Westbrook, 2002; Schoppa and Westbrook, 2001; Isaacson, 1999; Christie et al., 2005). Considering the anatomical and physiological distinctions between mitral and tufted cells, we asked whether one class of output neuron would be temporally coordinated with the other.

We obtained recordings from 24 spontaneously spiking pairs of mitral and middle-tufted cells with convergent dendrites, and in 20/24 pairs we detected at least one significant peak in the cross-correlogram. Figure 6 shows sample records and cross-correlograms from cell pairs in three empirical categories of correlated firing, similar to those observed in tufted-tufted pairs: slow correlation with a single broad central peak in the correlogram (Figs. 6B, 6C), a combination of slow and fast correlation with superposed broad and narrow peaks (Figs. 6D, 6E), and fast correlation with a single narrow peak (Figs. 6F, 6G). The fraction of pairs in each category was: broad peak only 7/20 (35 %), both broad and narrow peaks 11/20 (55 %), and narrow peak only 2/20 (10 %). Since broad and narrow peaks in cross-correlograms could occur either together or in isolation, we suspected that slow and fast correlations might be independent. Indeed, narrow peaks were observed in 2/6 pairs without broad peaks, and 11/18 pairs with broad peaks, rates not significantly different from those expected from an overall narrow peak fraction of 13/24 $\sim$ 50%, i.e. chance level ($P = 0.34$ and 0.24 respectively). Independence of slow and fast spike correlation was further supported by the lack of positive correlation between peak height and side area for correlograms with narrow peaks (Pearson's $R = -0.41$, $P = 0.12$, $n = 16$) (Fig. 4E).

In 3 mitral-tufted cell pairs, we could resolve the fine structure of the narrow peaks in cross-correlograms, using 1 ms time bins. Figures 7A and 7B show the 10 ms and 1 ms binned correlograms of one such pair. The peak was split into a pair of moderately narrow sub-peaks with positive and negative spike lags ($L = -7.8$ ms, $+8.4$ ms, widths $W = 8.9$ ms, 5.9 ms respectively). The sub-peaks reveal two modes of correlated firing at faster time scales, in which either the mitral cell fired ~ 10 ms before the tufted cell (negative lagged peak), or vice versa (positive lagged peak). In all recorded mitral-tufted pairs, we only found one with a sharp center peak representing a high precision temporal correlation ($W < 1$ ms).

Unlike tufted-tufted pairs, comparison of cross-correlogram peak lags and widths across mitral-tufted pairs did not reveal a prominent gap in the distribution of peak widths separating broad and narrow peaks (Fig. 7C). However it was still possible to classify peaks into two groups based on clustering in the width distribution: narrow peaks ($5 \text{ ms} < W < 50 \text{ ms}$) and broad peaks ($50 \text{ ms} < W < 3 \text{ s}$) (inset histogram of Fig. 7C). The two groups correspond to fast and slow modes of correlated firing in mitral-tufted pairs, separated at the same point (50 ms) as for tufted-tufted pairs. Narrow peak width was significantly smaller for tufted-tufted pairs (9.6 ± 9.1 ms, $n = 21$) compared to mitral-tufted pairs (15.1 ± 9.2 ms, $n = 16$) ($p = 0.04$, t-test, including sub-peaks). This was evident from the observation that 11/21 tufted-tufted peaks were narrower than 5 ms, compared to only 1/16 mitral-tufted peaks. Similarly, the mean unsigned lag of narrow peaks (unordered pairs) in mitral-tufted pairs (6.2 ± 3.6 ms, $n = 16$) was somewhat larger than in tufted-tufted pairs (4.3 ± 3.2 ms, $n = 21$) ($p = 0.046$, Mann-Whitney test). This indicates a more precise correlation in tufted-tufted cell spiking. The mean signed lag of narrow peaks for ordered mitral-tufted pairs was not significantly different from zero (t-test, $p = 0.313$, $n = 16$), so that either negative or positive lags were equally likely, and either mitral or tufted cell could fire first and lead the other during correlated spiking (Fig. 7D).

As in tufted-tufted pairs, correlated firing of mitral-tufted pairs was related to the convergence or divergence of apical dendrites. Of 24 pairs with convergent dendrites, a broad peak was present in 18 pairs (75%), significantly greater than chance ($P = 0.011$); a narrow peak was present in 13 pairs (54%), which was at chance level ($P = 0.42$); and 0/7 pairs with divergent dendrites had any peaks, much less than chance level ($P = 0.004$). Thus, there was an elevated probability of observing slow correlated firing in both mitral-tufted and tufted-tufted pairs with convergent dendrites. In tufted-tufted pairs, the probability of fast correlated firing (narrow peak) was significantly greater than for mitral-tufted cell pairs. To further assess glomerular projection as a determinant of correlated spiking, we compared side areas and center heights of cell pairs with convergent or divergent primary dendrites (Figs. 7E, 7F). For divergent dendrites, side area was not significantly different from zero (t-test, $p = 0.807$, $n = 7$), whereas for pairs with convergent dendrites, it was significantly greater than zero ($p = 0.0055$, $n = 18$ correlograms with broad peaks). Center heights of correlograms with significant narrow peaks were greater for pairs with convergent dendrites ($n = 13$) than for pairs with divergent dendrites ($n = 7$) ($p = 0.00057$, Mann Whitney test). The maximum value of the cross correlogram in the interval ± 100 ms was not significantly greater than $1 + 3 \times \text{SD}$ for pairs with divergent dendrites (paired t-test, $p = 0.936$ for SD from shuffled cross correlogram, 0.964 for SD from Poisson distribution, $n = 8$), but was greater for pairs with convergent dendrites ($p = 0.0117$ for shuffled SD, 0.0123 for Poisson SD, $n = 18$). Taken together, these results suggest that the firing correlations seen in mitral-tufted pairs, on both slow and fast time scales, also rely on intraglomerular or close interglomerular couplings.

As in tufted-tufted pairs, the regularity of firing patterns, quantified by interspike CV, was correlated for mitral and tufted cells with convergent dendritic projections ($R = 0.49$, $P = 0.015$, $n = 24$) (Fig. 7H). However, there was poor correlation for the burst fraction ($R =$

-0.45 , $P = 0.27$, $n = 8$ pairs) and the mean burst rate ($R = -0.38$, $P = 0.35$, $n = 8$ pairs), so the coupling between mitral and tufted cells may be weaker than that between pairs of tufted cells. This was consistent with the lack of a strong relation between the presence and absence of bursting in convergent mitral and tufted cells: there were 7 non-bursting pairs, 9 pairs with one bursting cell, and 8 pairs with both cells bursting, a random distribution ($P = 0.15$). For divergent pairs, bursting also occurred at chance level: 3/7 divergent mitral-tufted pairs were single burster pairs ($P = 0.50$).

In summary, both tufted-tufted and mitral-tufted cell pairs exhibited independent fast and slow correlations in spiking (narrow and broad correlogram peaks), that were only manifested when the primary dendrites converged and overlapped in the glomerular layer. Narrow peaks sometimes resolved into sub-peaks, indicating multiple modes of fast correlated firing. The peak widths, lags and firing statistics suggested a tighter coupling in tufted-tufted pairs.

Dependence of correlated firing on bursting

Bursting imposes a temporal structure on spike trains and might therefore influence temporal correlations of firing across cells. Slow correlations in spiking could emerge from temporal alignment of bursts, and we predicted that the occurrence of broad peaks in the cross-correlogram should be related to the bursting status of cells. This was true in tufted-tufted pairs, in which broad peaks were found in 10/10 pairs with both cells bursting, but only 3/8 pairs with only one, or neither member bursting ($P = 0.04$ in both cases, deviating from overall frequency of $13/18 = 72\%$ broad peaks). In mitral-tufted pairs, the corresponding broad peak fractions were 8/8 and 10/16 pairs, not significantly different from the overall frequency of $18/24 = 75\%$ broad peaks. This again suggests a tighter coupling of tufted-tufted pairs than mitral-tufted pairs. For narrow peaks, there was no relation between peak occurrence and bursting status ($P > 0.25$, computed with overall frequency of narrow peaks 78% for tufted-tufted and 50% for mitral-tufted pairs), as expected if slow and fast correlations were independent. We also investigated whether the strength or temporal precision of correlations was related to bursting, and found that amplitudes, widths and lags of broad or narrow peaks did not differ significantly across 3 groups of cell pairs (with 0, 1 or 2 bursting cells) (KS tests; $P > 0.10$).

Previously, it has been shown in ET cells that the strength of slow correlated firing is inversely related to the bursting frequency (Hayar et al., 2004a). We therefore sought to determine whether mean spike rate, mean burst rate and other burst-related parameters exerted any influence on correlated firing in middle tufted and mitral cells. A set of parameters characterizing spiking and bursting patterns (CV, mean burst rate, burst fraction, interburst CV, intraburst spike rate, intraburst spike CV, mean number of spikes per burst, mean burst duration; mean intraburst ISI, and burst duration CV) was compared for subsets of pairs with and without broad peaks or narrow peaks, assuming independence of broad and narrow peaks. In mitral cells, there was no significant difference in parameters related to the presence or absence of broad and narrow correlation peaks (KS tests, $P > 0.10$). In tufted cells that were members of mitral-tufted pairs, spike CV was significantly higher for those with broad peaks, than those without (KS test, $P < 0.025$; 18 cells in pairs with broad peak, 6 cells in pairs without). This is consistent with the CV being higher for irregularly firing cells with clustered spikes, such as bursting cells, so that slow correlations (broad peaks) emerge more readily by temporal alignment of clusters. We also found that burst fraction and mean spikes per burst (both measures of bursting intensity) were significantly higher for pairs without narrow peaks, than with narrow peaks (KS test, $P < 0.05$; 8 cells in pairs with narrow peak, 5 cells in pairs without), suggesting that fast spiking correlations tended to occur more in pairs that were less intensely bursting.

In tufted cells that were members of tufted-tufted pairs, spike CV was also significantly higher for those with broad peaks, than those without (KS test, $P < 0.025$; 26 cells in pairs with broad peak, 10 cells in pairs without), in agreement with the result from mitral-tufted pairs. We could not test the difference between spike bursting parameters of tufted-tufted pairs with and without broad peaks because, of 12 pairs with at least one bursting cell, all but one had a broad peak. For narrow peaks, the burst fraction was significantly higher for cells in pairs that did not have a narrow peak (KS test, $P < 0.025$; 16 cells in pairs with narrow peak, 6 cells in pairs without). In tufted-tufted pairs the strength of fast correlations, measured by heights of narrow peaks in the cross correlogram, was inversely related to burst fraction ($R = -0.42$, $P = 0.03$) and mean burst rate ($n = 26$ cells in 13 bursting pairs). This further supports the occurrence of more fast spiking correlations in less intensely bursting tufted cell pairs. A similar result was not obtained for mitral-tufted pairs. In contrast to ET cells, we did not find an inverse correlation between slow spiking correlation (broad peak height) and burst rate for either mitral-tufted or tufted-tufted pairs.

In summary, broad cross correlogram peaks were related to spike CV and bursting in tufted cells, consistent with correlated bursting in cell pairs. Conversely, narrow cross correlogram peaks appeared to be inversely related to bursting. The latter result suggests that once a burst has been initiated, spiking is determined more by intrinsic membrane conductances of the cell, than by fast correlating inputs from other coupled cells (Chen and Shepherd, 1997; Desmaisons et al., 1999; Balu et al., 2004).

Role of AMPA receptors in correlated firing

Ionotropic glutamate receptors have been implicated in the slow firing correlations of ET cells and in the fast correlations of mitral cells connected to the same glomerulus (Schoppa and Westbrook, 2002; Hayar et al., 2005). In particular, AMPA receptors may be responsible for generating correlated synaptic potentials in the apical dendritic tufts of principal neurons. To test whether AMPA receptors are required for establishing correlated firing in tufted cells, we applied the AMPA receptor antagonist NBQX (20 μ M) to slices by extracellular perfusion while recording spontaneous spike activity in cell pairs with convergent dendrites.

First, we determined the actions of NBQX on the basal firing of mitral and tufted cells. In tufted cells, we saw variable effects of NBQX. Figure 8 compares spike recordings and associated ISI distributions, joint ISI histograms and IBI and intraburst frequency distributions from a bursting tufted cell obtained under control conditions, and after introducing NBQX. It is apparent that in NBQX, the burst firing of this cell was intensified: CV, burst fraction, mean spikes per burst and mean burst duration were all substantially boosted. Regularity of bursting was improved (interburst CV decreased), spiking within bursts also became more regular (intraburst CV decreased), and the joint ISI histogram reorganized into compact, cleanly separated clusters. Although similar effects were seen in two additional tufted cells, the spike patterning of 10 other tufted cells, both bursting and non-bursting, was not significantly altered in NBQX. Figs. 9A–9F show an example of spike records, ISI distributions and joint ISI histograms of a non-bursting tufted cell that was not changed by NBQX perfusion. Changes in several spiking parameters in NBQX are summarized as ratio plots in Figs. 9G–9K (filled circles), and we also plotted spontaneous shifts in the same parameters without drug application, for control cells followed over time intervals comparable to the time course of NBQX perfusion (ca. < 400 s prior to drug, > 400 s after drug) (open circles). The latter show the normal drift in spiking behavior over the extended recording periods of drug perfusion experiments. In group comparisons with these control data, the spread in burst fraction, mean spike rate, mean burst rate, interburst CV, mean intraburst spike rate, intraburst CV, mean spikes per burst and mean intraburst ISI were not significantly different in NBQX. However, we found that in NBQX there was a

significant elevation in group average CV ($P < 0.005$) and mean burst duration ($P < 0.01$) (KS tests), due to a few cells whose bursting was strongly accentuated in NBQX (e.g. Fig. 8; data points marked by asterisk in Figs. 9J, 9K). Thus, tufted cells in slices appear to be heterogeneous in their sensitivity to NBQX, and AMPA receptor-mediated synaptic transmission may make firing more irregular in a minority of cells.

In mitral cells, NBQX did not cause the same degree of enhancement of bursting activity that was observed in some tufted cells. In Figs. 10A–10J, we compare spike recordings, ISI distributions, joint ISI histograms and IBI and intraburst frequency distributions from a typical bursting mitral cell under control conditions and in NBQX. In contrast to the tufted cell of Fig. 8, this bursting mitral cell was relatively unaffected by NBQX. Group effects of NBQX on selected mitral cell spiking and bursting parameters are shown in ratio plots (Figs. 10K–10O, filled circles), along with spontaneous drifts in a control group of cells (open circles). In group comparisons, there were no significant differences attributable to NBQX for: spike CV, burst fraction, mean spike rate, mean burst rate, interburst CV, mean intraburst spike rate, intraburst CV, mean spikes per burst, mean burst duration, and mean intraburst ISI (KS tests, $P > 0.10$).

Next, we examined the effects of NBQX on the heights and widths of cross-correlogram peaks to determine whether AMPA receptors play a role in correlated firing. Figure 11A shows split narrow peaks in the spike cross-correlogram of a tufted-tufted cell pair before and after application of NBQX. In the presence of the drug, both split peaks persisted, with similar amplitudes, and similar or narrower widths. An 11 additional narrow peaks or sub-peaks from 7 tufted-tufted pairs all persisted to varying degrees in NBQX (normalized ratios plotted in Fig. 11B). When we compared peak height and width changes in NBQX (filled circles) with changes due to spontaneous drift in a set of control recordings of similar duration (open circles), we found no significant differences between the two groups (KS tests, $P > 0.10$). In particular, 4 sharp peaks representing high precision fast correlation ($1 < W < 2.5$ ms) were still present in NBQX, and were either unchanged or partially reduced (H ratios: 1.18, 0.78, 0.63, 0.37). A similar approach was used to analyze the effect of NBQX on broad correlogram peaks, corresponding to slow firing correlations (Figs. 11C–11D). Again, a group comparison with controls did not detect significant differences in either broad peak height or width related to NBQX.

We noticed a few outliers among tufted cell peaks tested with NBQX, including one with a large increase in amplitude (Fig. 11B, left panel) that was a part of a triad of split central peaks in one cell pair (the other two peaks in the same cross-correlogram were nearly unchanged in NBQX). In this isolated case, it is not possible to know if the increase was due to NBQX, or to spontaneous changes in the glomerular network. However, the same pair also showed a strong increase in broad peak height in NBQX, suggesting that some aspect of correlated firing in this pair may have been under the control of AMPA receptors. If we excluded the unusual peak with large height increase (Fig. 11B), then there was no correlation between the NBQX ratios for peak height and burst fraction ($P > 0.10$, $n = 16$ peaks), i.e. no evidence for a relation between fast correlation and burst firing. If NBQX strengthens bursting in some tufted cells (Fig. 8), and if fast correlations tend to be weaker in bursting cells, we might expect narrow peaks to be more sensitive to NBQX in the presence of broad peaks (which can emerge from correlated bursting). However, we did not detect any significant differences in narrow peak height and narrow peak width ratios between correlograms with and without broad peaks (KS test, $P > 0.10$, comparing 6 pairs with broad peaks to 6 without). Similarly, there was no effect of bursting status on the peak height and width ratios (KS tests, $P > 0.10$, comparing 5 non-bursters to 19 bursters). This is consistent with independent mechanisms for fast and slow correlations.

In mitral-tufted pairs, there was also no significant effect of NBQX on heights and widths of either narrow or broad peaks in group comparisons (KS tests, $P > 0.10$) (Figs. 11E–11H). However, we did notice a marked increase (> 2 -fold) in the heights of 2/5 broad peaks (Fig. 11H), which corresponded to: (i) a pair in which non-bursting mitral and tufted cells transitioned to bursting after applying NBQX, and (ii) a pair in which both mitral and tufted cells showed a > 1.7 -fold increase in spike CV after applying NBQX. In a minority of cells with bursting regulated by AMPA receptors, NBQX could strengthen slow correlations by promoting bursting. However, as in tufted-tufted pairs, we did not detect any relation between the effects of NBQX on peak height and width, and bursting status of either the mitral or tufted cell in the pairs (KS tests, $P > 0.10$; non-bursters compared to 5 bursters for narrow peaks; 4 non-bursters compared to 6 bursters for broad peaks). Finally, we tested whether the overall level of spike activity (mean spike rate) was correlated with actions of NBQX. Higher spike activity could intensify glutamatergic lateral excitation between intraglomerular dendritic tufts, perhaps increasing intercellular coupling by AMPA receptors as a mechanism for spike correlation. However, we found no correlation of narrow and broad peak height and width ratios with mean spike rate, either for tufted-tufted ($P > 0.05$) or mitral-tufted pairs ($P > 0.20$) (least squares regressions).

In summary, we found that the AMPA receptor antagonist NBQX did not abolish fast and slow firing correlations in tufted-tufted and mitral-tufted pairs. Either the changes in cross-correlogram peaks in NBQX were not significantly different from the control group, or there was actually an increase in peak height for a minority of pairs. The latter may represent subsets of either mitral or tufted cells in which AMPA receptor blockade promotes bursting and improves slow correlations. The variability of firing during protracted recording in drug perfusion experiments could have obscured more subtle effects of NBQX, so we cannot rule out some contribution of AMPA receptors to correlated firing. However, it is clear that robust fast and slow correlated firing can be maintained even under AMPA receptor blockade.

Since our findings from tufted cells differed from previous reports suggesting that AMPA receptors are required to establish fast correlations (< 5 ms) in mitral cell spiking (Schoppa and Westbrook, 2002), we performed control experiments to verify that bath-applied NBQX could in fact penetrate glomeruli and reach AMPA receptors on tufted cell distal dendrites. With 1 mM MNI-caged glutamate in the perfusion solution, we used flash photolysis to uncage glutamate onto dendritic tufts while recording whole-cell currents from either mitral or tufted cells with membrane voltage held at -60 mV (Lowe, 2003). We found that NBQX was able to abolish fast excitatory postsynaptic currents activated by uncaging glutamate. Our drug application protocol for slices was identical to that of a previous study, in which we successfully blocked intraglomerular AMPA receptors of external tufted cells (Ma and Lowe, 2007).

Electrical coupling in tufted cells

Electrical coupling through gap junctions has been implicated in the correlations of slow bursting of external tufted cells (Hayar et al., 2005) and fast spiking of mitral cells connected to the same glomerulus (Christie et al., 2005). We were able to obtain double whole-cell recordings from several tufted-tufted or mitral-tufted pairs, and tested for electrical coupling. The voltage coupling coefficient (ratio of voltage in the output cell to voltage in the input cell) was computed either from voltage-clamped currents induced by stepping the membrane potential of one cell from -60 mV to -200 mV (Figs. 12A, 12B), or from voltage deflections induced by injecting hyperpolarizing current into either cell (Figs. 12C, 12D). Electrical coupling was detected in 4/4 tufted-tufted pairs with convergent dendrites, with mean coupling coefficient 0.046 ± 0.012 (mean $\pm$ SEM, $n = 6$, average including forward and reverse coupling measurements, range 0.008–0.086). Coupling was

also detected in 7/7 mitral-tufted pairs with convergent dendrites. The mitral-to-tufted forward coupling coefficient (i.e., ratio of tufted voltage to mitral voltage) was 0.021 ± 0.00421 (mean $\pm$ SEM, $n = 6$, range 0.010–0.035), and the tufted-to-mitral (reverse) coupling coefficient was 0.016 ± 0.00236 (mean $\pm$ SEM, $n = 7$, range 0.010 – 0.027). In our sample, the forward and reverse couplings were not significantly different ($p = 0.19$, paired t-tests, $n = 6$), and pooling forward and reverse values yielded a mean of 0.018 ± 0.002 ($n = 13$). An unpaired t-test comparing tufted-tufted couplings to mitral-tufted couplings, averaging forward and reverse coefficients of mitral-tufted pairs to remove pair correlations, suggested that tufted-tufted coupling might be stronger than mitral-tufted coupling ($p = 0.03$, 9 degrees of freedom). Additional data from larger samples will be needed to rigorously test this hypothesis. The overall range of values of coupling coefficients we found (~ 0.01 – 0.1) was comparable to ranges previously reported for mitral-mitral pairs (Schoppa and Westbrook, 2002; Christie et al., 2005; Christie and Westbrook, 2006; Pimentel and Margrie, 2008). The existence of electrical synapses between tufted cells, or tufted and mitral cells, provides a potential mechanism for mediating slow and fast spike correlations independent of AMPA-receptor mediated synaptic transmission.

DISCUSSION

Different firing patterns of mitral/middle tufted cells and ET cells

Our recordings of spontaneous activity from mitral and middle tufted cells in olfactory bulb slices revealed a wide range of spiking patterns, including random Poisson firing, intermittent bursting and more regular, rhythmic bursting. The spiking was different from the bursting activity seen in another class of olfactory bulb principal neurons, the external tufted (ET) cells (Hayar et al., 2004b). Compared to mitral and tufted cells, ET cells exhibited a higher mean burst rate (3.3 ± 0.8 , 5.5–5.7 times larger), lower mean spikes per burst (5.4 ± 0.3 , 0.67 times smaller), shorter mean intraburst ISI (8.4 ± 0.5 ms, 0.17–0.20 times smaller), shorter mean burst duration (32 ± 2.4 ms, 0.11–0.12 times smaller), and reduced mean interburst CV (0.43 ± 0.03 , 0.41 times smaller). Thus, ET cells burst more rapidly and regularly than mitral and tufted cells, with shorter bursts and higher intraburst spike frequency. In ET cells, bursting becomes more regular at higher burst rates, reflected by a decrease of interburst CV and increase in autocorrelation peak amplitude (Hayar et al., 2004b). We did not find a correlation between interburst CV and burst rate in tufted cells ($R = 0.18$, $P = 0.22$, $n = 49$), although there was a moderate inverse correlation in mitral cells ($R = -0.61$, $P = 0.004$, $n = 20$). Due to their irregular bursting, an autocorrelation peak was present in only 4/20 mitral cells and 15/49 tufted cells, and there was no significant relation between autocorrelation peak amplitude and mean burst rate ($P = 0.35$ and 0.13 respectively). These differences from ET cells might be attributable to stronger and more complex inhibitory synaptic regulation of mitral and tufted cells, which appear to be positioned downstream from ET cells in the glomerular network (Gire and Schoppa, 2009; De Saint et al., 2009).

Glomerulus-specific correlation of mitral and middle tufted cell firing

Temporal correlations were present in the spiking of both tufted-tufted and mitral-tufted cell pairs with convergent glomerular projections. Spike cross-correlograms contained discrete central peaks with a bimodal width distribution, corresponding to slow (> 50 ms) and fast (< 50 ms) firing correlations, and the precision of fast correlations varied widely from ca. 1 ms to 30 ms. Our results extend previous findings from mitral-mitral cell pairs, which also exhibit glomerulus-specific spike correlations on fast and slow timescales (Christie et al., 2005; Isaacson, 1999; Schoppa and Westbrook, 2001; Schoppa and Westbrook, 2002). Thus, temporal coordination of output signaling exists both within and between the two types of principal neurons linked through a glomerulus. The linkages appear to be somewhat tighter

in tufted-tufted pairs, which had smaller mean width and lag of narrow peaks in cross-correlograms, significantly correlated firing or bursting patterns, and perhaps stronger mean electrical coupling. In contrast to previous studies of evoked responses of mitral and tufted cells in vivo (Nagayama et al., 2004; Griff et al., 2008), we did not detect significant differences between basal spontaneous firing rates of mitral and tufted cells in slices. Thus, the two cell types may express divergent spike activity only when relaying signals that encode odorant stimuli.

Slow correlations in firing may be related to the low frequency (< 10 Hz) glomerulus-specific burst firing of mitral cells driven by long lasting depolarizations (Isaacson, 1999; Schoppa and Westbrook, 2001; Carlson et al., 2000; Puopolo and Belluzzi, 2001). Correlated slow rhythmic activity of neurons connected to a glomerulus may be coordinated by the intrinsic bursting of ET cells (Hayar et al., 2004a; Hayar et al., 2004b; De Saint et al., 2009; Liu and Shipley, 2008). It has been suggested that this gates glomerular output relative to periodic sensory input generated when an animal samples its olfactory environment by sniffing (Wachowiak and Shipley, 2006; Zhou and Belluscio, 2008). Sniff-wise temporal coordination of olfactory processing would be expected if a single sniff encompasses the full computation cycle required for odorant recognition and discrimination (Uchida and Mainen, 2003; Rinberg et al., 2006b; Wesson et al., 2008). Our results indicate that this slow rhythm can regulate the output of the middle tufted cells. It is likely that all principal cells relaying output from an individual glomerulus would be organized into slow coordinated assemblies. Tufted cells are heterogeneous, and other sub-types such as superficial tufted cells located immediately beneath the glomerular layer can have distinctive spiking properties (Griff et al., 2008). Additional studies are needed to verify that other sub-types of tufted cells are also tied into the slow oscillatory network

AMPA receptors are not required for correlated firing in middle tufted cells

What mechanisms are responsible for glomerulus-specific correlated firing in tufted cells? In mitral cell pairs, the electrical coupling of AMPA autoreceptor potentials between mitral cell dendritic tufts was proposed to mediate glomerulus-specific spike correlation on fast time scales of a few ms (Schoppa and Westbrook, 2002). Our data do not support this as an exclusive mechanism for either tufted-tufted or mitral-tufted pairs, because blockade of AMPA receptors by NBQX failed to abolish spike correlation on either fast or slow time scales. By the same token we can also rule out a requirement for conventional AMPA receptor-mediated transmission between tufts (Pimentel and Margrie, 2008; Urban and Sakmann, 2002; Christie and Westbrook, 2006), AMPA receptor-mediated excitation of interneurons that feed temporally correlated inhibitory inputs to tufted cells (Hayar et al., 2005), and shared excitatory synaptic input from olfactory receptor nerve terminals (Kazama and Wilson, 2009) mediated by AMPA receptors. Since the cells in our experiments were active at low basal spike frequencies (< 10 Hz), we do not exclude the possibility that AMPA receptor-mediated transmission could strengthen spike correlations at higher frequencies. At present, we cannot exclude the possibility that GABAergic inhibitory inputs or excitatory inputs mediated by NMDA receptors may contribute to correlated firing of tufted cells. Coordinated GABAergic and glutamatergic synaptic transmission has been implicated in slow correlations of ET cells (Hayar et al., 2005). We were not able to pharmacologically test the requirement for NMDA receptors, because antagonism of these receptors silenced spontaneous spike activity of mitral and tufted cells in our preparations.

Synchronization of mitral/tufted cells by gap junctions

We found that both tufted-tufted and mitral-tufted pairs are coupled electrically, and hence gap junctions are a potential mechanism underlying the spike correlations. Knockout of connexin 36, a gap junction protein expressed in glomeruli, disrupts millisecond spike

correlations in mitral cell pairs (Christie et al., 2005). Electrical coupling has been documented between homotypic output neurons of *Xenopus* olfactory bulb, and their spiking is correlated with peak widths of ca. 14 ms, the same time scale as our moderately narrow peaks from tufted cells (Chen et al., 2009). Gap junctions between apical dendritic tufts of mitral or tufted cells have been identified in serial electron microscopic reconstructions of mouse glomeruli (Kosaka and Kosaka, 2004), and identified as connexin 36 by immunogold labeling in mice and rats (Christie et al., 2005; Rash et al., 2005). They appear to connect mitral/tufted dendrites through either direct contacts, or indirect pathways routed through inter-neuronal processes (Kosaka and Kosaka, 2005).

The distal location of these junctions relative to the mitral/tufted cell somata raises the question of how effectively they could couple and synchronize spiking in connected cells. An action potential initiated at the soma of one mitral/tufted cell can backpropagate up to the apical tuft (Bischofberger & Jonas, 1997; Debarbieux et al., 2003), where it would electrically couple to the tuft of a second cell. The impact of this coupled fast spike potential on the soma of the second cell will depend on its filtering by membrane capacitance (Bennett & Zukin, 2004). If it is slowed and attenuated by passive conduction down the primary dendrite, spike initiation in the second cell may fail or be delayed. This might suggest that the coupling of slower AMPA autoreceptor potentials will be more effective for generating correlated spiking in connected pairs (Schoppa and Westbrook, 2002). However, a critical factor is the active membrane of the primary dendrite and glomerular tuft, which is furnished with a high density of Na^+ channels that support distal dendritic initiation and forward propagation of action potentials (Chen et al., 1997; Chen et al., 2002; Djurisic et al., 2004; Zhou et al., 2006). Computational models of mitral cell pairs with active dendrites and tufts coupled solely by gap junctions, can generate correlated spiking that is synchronized on fast (< 5 ms) time scales, without the participation of AMPA receptors (Migliore et al., 2005). This is consistent with our data showing robust correlated firing under AMPA-receptor blockade.

Mechanisms of multiple correlogram peaks

Our finding of broad, moderately narrow, and high precision cross-correlogram peaks in tufted and mitral cell pairs with convergent glomerular projection invites comparison with similar broad, medium and narrow correlations observed in spatially proximate retinal ganglion cells (Mastronarde, 1989; Brivanlou et al., 1998). Like tufted cells, ganglion cell correlations also occurred spontaneously in the absence of external stimulation (i.e., in the dark). At medium or narrow time scales, they are mediated by gap junction couplings shared between ganglion cells and amacrine cells, or directly between ganglion cells. The presence of double off-center correlation peaks at medium time scales was explained in terms of waves of excitation propagating across the retina in opposing directions. Suppose for the sake of argument that gap junctions are the primary mechanism underlying tufted-tufted and mitral-tufted spike correlations. Fig. 13 illustrates schemes of electrical network excitation that could account for the various cross-correlograms structures we observed. By analogy with the retina, moderately narrow double peaks in spike cross-correlograms could be interpreted as bidirectional spread of spontaneous excitation through an electrically coupled network of principal neurons within a glomerulus (Fig. 13C). A single off-center peak might correspond to unidirectional propagation of spike activity through the network, with reverse propagation paths being blocked by asymmetric coupling due to unequal input impedances (Fig. 13B). Sharp peaks ($W < 2$ ms) always exhibited short spike lags ($L < 3$ ms), and some of these may correspond to direct electrical connections between two recorded cells (Fig. 13D). Moderately narrow peaks ($5 \text{ ms} < W < 30 \text{ ms}$) with longer spike lags ($5 \text{ ms} < L < 13 \text{ ms}$) could reflect multicellular electrical pathways linking the cell pairs (Fig. 13A). This is consistent with the tendency for peak width to increase with peak lag in a subset of the

narrow peaks (Figs. 5C, 7C). Voltage fluctuations in linking cells would contribute jitter to polysynaptic relays and broaden correlation peaks. The mean lag of peaks wider than 2 ms was 5.7 ± 3.3 ms ($n = 32$), so assuming that directly coupled cells have a lag of ca. 1–2 ms, the shortest paths between randomly selected mitral-tufted or tufted-tufted pairs may include fewer than 3 – 6 electrical relays between principal neurons.

Some narrow cross-correlogram peaks resolved into multiple sub-peaks that included a sharp central peak as well as one or more moderately narrow off-center peaks (see Figs. 5B, 11A). These might arise from propagation of excitation through multiple electrical pathways between cell pairs: off-center peaks from delayed excitation through multicellular routes, and sharp central peaks by either direct coupling between the recorded cells, or direct coupling of both cells to another excitatory cell (Fig. 13D). The *L-W* plots in Figs. 5C and 7C reveal subsets of narrow peaks with near zero lags (< 1 ms) but widths larger than 10 ms. These can arise if the two recorded cells are excited by a third cell through symmetric multicellular connections.

Multiple sub-peaks might also reflect multiple modes of spike initiation in coupled cells: (1) a sharp central peak may arise when one or both cells initiate action potentials in their dendritic tufts, which then propagate forward to their somata; (2) broader, off-center peaks may arise when an action potential in either cell triggers a delayed spike in the other cell, whose initiation site is shifted to the primary dendritic trunk or soma following a subthreshold conduction delay introduced by capacitive filtering of the dendrite. The tendency for tufted-tufted pairs to exhibit stronger and tighter spike correlations than mitral-tufted pairs may then be explained by heavier filtering of electrically coupled potentials by the longer primary dendrites of mitral cells. In this scenario, the relative frequency and efficiency of somato-dendritic versus tuft initiation would determine the respective heights of off-center and on-center sub-peaks, that would vary with dendritic geometry and modulation by spontaneous synaptic inhibition. Somato-dendritic inhibition from granule cells can regulate the spike initiation site, shifting it up to the apical tuft (Chen et al., 1997; Djuricic et al., 2004). A background barrage of spontaneous inhibitory synaptic potentials may randomly shift the initiation site, introducing variable delays and broadening of the off-center correlogram sub-peaks. Given the high levels of tonic activity of the mitral/tufted – granule cell network in awake, behaving animals (Davison and Katz, 2007; Doucette and Restrepo, 2008; Kay and Laurent, 1999; Rinberg et al., 2006a), we propose that in the normal basal state of the olfactory bulb circuit, strong tonic inhibition from granule cells constrains spike initiation to the apical tuft, thereby maintaining high precision (millisecond) intraglomerular spike synchrony of output neurons by default. Strong tonic granule cell activation may also promote, by correlated asynchronous feedback, precise inter-glomerular spike synchrony in the basal state (Marella and Ermentrout, 2010).

Connectivity of mitral/tufted cells in gap junction networks

Although electrical coupling was detected in all cell pairs tested, we found a roughly 10-fold variation of voltage coupling coefficients ($\sim 0.01 - 0.1$). Some of this variability may be due to variation in cell morphology and resting membrane conductance. In the electrical network models (Fig. 13), stronger couplings might also correspond to direct electrical connections between recorded cells, and weaker couplings to indirect linkages via one or a few interpolated cells. If each glomerulus included ca. 20 mitral cells and as many tufted cells (Royet et al., 1998), then a total of ca. 40 principal neurons are wired together with relatively short minimal paths (< 6 linkages) between cell pairs. Tufted-tufted pairs had significantly shorter narrow peak lags than mitral-tufted pairs, and possibly stronger electrical coupling, suggesting that tufted cells may preferentially cluster together in the network. It is notable that the fraction of pairs with narrow peak lags of 3 ms or shorter, that might be due to direct coupling or separation by one interpolated cell, was a substantial

7/21(33.3 %) for tufted-tufted pairs, but only 1/16 (6.3 %) for mitral-tufted pairs. Short connection paths are compatible with non-random connectivity in networks with a small-world architecture (Watts and Strogatz, 1998). The diversity of narrow peak structures in cross-correlograms suggests that not all tufted-tufted or mitral-tufted pairs are equally connected. Some cells may have greater connectivity than others, and act as hubs in the network. A small-world network can promote moderately fast temporal correlation and binding of spike outputs in all principal neurons of a glomerulus, and still allow functional separation of mitral and tufted cells to perform different olfactory processing tasks.

Function of temporally correlated firing

An olfactory bulb glomerulus is a fundamental unit of sensory coding, receiving input from a unique olfactory receptor tuned to recognize specific odorant molecular features. During odor perception, input signals from a large repertoire of distinct olfactory receptors are processed by a massively parallel array of glomeruli, and output signals are relayed to cortex via multiple channels of mitral and tufted cell spike activity. The transformation from input to output depends on a complex network of bulbar interneurons. Synaptic pathways in this network can cross-couple principal neurons belonging to different glomeruli, combining signals from different receptor-coded channels representing different molecular features. Further opportunities for combinatorial processing of receptor codes exist in the distributed, overlapping projections of mitral or tufted cells in olfactory cortex (Stettler and Axel, 2009). Downstream neurons and circuits face the task of decoding and interpreting mixed signals from multiple glomeruli, a problem that might be solved by temporal correlations across parallel spike channels. This would preserve the integrity of the glomerulus as a functional unit of molecular feature coding (Chen et al., 2009). Glomeruli may split olfactory receptor-coded information into distinct parallel streams conveyed by mitral and tufted cells (Ezeh et al., 1993; Griff et al., 2008; Macrides et al., 1985; Nagayama et al., 2004; Schneider and Scott, 1983). We show that these parallel streams can be temporally correlated, even if they encode different aspects of odor stimuli.

Synchronization by a hard-wired intraglomerular network of electrical contacts ensures that glomerulus-specific spike correlation can be maintained over a wide range of activity levels, independent of evoked chemical synaptic transmission. In our slice experiments, correlations were evident at low spontaneous spike rates (< 10 Hz) comparable to basal spike rates of mitral/tufted cells in anesthetized animals. We expect that this temporal binding will also be available to glomeruli at higher frequencies of basal firing (~ 10 – 30 Hz) characteristic of mitral/tufted cells in awake behaving animals (Davison and Katz, 2007; Doucette and Restrepo, 2008; Kay and Laurent, 1999; Rinberg et al., 2006a), and will remain operational as the basal spike rate is modulated up or down by odorant stimuli. Temporal binding of both mitral and tufted cell output from individual glomeruli provides a framework for combinatorial integration of odor representations. For example, higher order perceptual objects composed of different molecular features in an odor ‘scene’ could be constructed if assemblies of neurons with multiple glomerular projections were bound through specific lateral inhibitory connections (Galán et al., 2006; Kashiwadani et al., 1999; Mori et al., 1999; Schoppa, 2006; Wilson and Stevenson, 2006; Willhite et al., 2006). Correlated activity across glomeruli might also play a role in olfactory memory, promoting long term potentiation of synapses and inscribing odor memories into the olfactory bulb network. Such strengthened synapses could facilitate temporal binding and recognition of memorized constructs with lower stimulus thresholds.

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Abbreviations

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CV	coefficient of variation
EPL	external plexiform layer
ET	external tufted
GCL	granule cell layer
GL	glomerular layer
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IB	intraburst
IBI	interburst interval
ISI	interspike interval
KS test	Kolmogorov–Smirnov test
MCL	mitral cell layer
MNI	4-methoxy-7-nitroindolyl
NBQX	(±)-4-(4-Aminophenyl)- 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide
SD	standard deviation
SEM	standard error of the mean

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Figure 1. Bursting and non-bursting spiking patterns of tufted cells

A–E. Tufted cell with strongly bursting spontaneous spike activity.

A. Segment of whole-cell current clamp recording of membrane voltage (20 s) with detected burst spikes indicated above in a raster plot of spike times.

B. Linear-log plot of interspike interval (ISI) distribution for entire record (200 s, 1066 spikes) showing bimodal distribution. Inset: log-linear plot showing non-exponential distribution. Arrow: bursting threshold (75 ms).

C. Plot of joint ISI distribution of all spikes. Gray lines: 75 ms ISI threshold; open circles: burst spikes; filled circles: non-burst spikes.

D. Plot of the distribution of interburst intervals (IBI) (from 159 bursts). Inset: log-linear plot, showing nearly exponential distribution.

E. Plot of distribution of instantaneous intraburst spike frequencies (from 738 intraburst ISIs) *Parameters:* mean spike rate 5.32 Hz, coefficient of variation (CV) 2.01, fraction of spikes in bursts 0.85, mean burst rate 0.80 Hz, interburst CV 0.85, mean spikes per burst 5.6, mean burst duration 180 ms, burst duration CV 0.63, mean intraburst ISI 39.8 ms, mean instantaneous intraburst spike rate 26.7 Hz, intraburst CV 0.25.

F–H. Tufted cell with non-bursting spontaneous spike activity.

F. Segment of a cell-attached voltage clamp recording of action currents (length 56 s).

- G.** Linear-log plot of interspike interval (ISI) distribution for entire record (400 s, 315 spikes) showing unimodal distribution. Inset: log-linear plot showing exponential (Poisson) distribution.
- H.** Plot of joint ISI distribution of all spikes. *Parameters:* mean spike rate 0.79 Hz, CV 1.02.

Figure 2. Bursting and non-bursting spiking patterns of mitral cells

A–E. Mitral cell with strongly bursting spontaneous spike activity.

A. Segment of cell-attached voltage clamp recording of action currents (20 s) with detected burst spikes indicated above in a raster plot of spike times.

B. Linear-log plot of interspike interval (ISI) distribution for entire record (200 s, 1043 spikes) showing bimodal distribution. Inset: log-linear plot showing non-exponential distribution. Arrow: bursting threshold (75 ms). **C.** Plot of joint ISI distribution of all spikes. Gray lines: 75 ms ISI threshold; open circles: burst spikes; filled circles: non-burst spikes.

D. Plot of the distribution of interburst intervals (IBI) (from 127 bursts). Inset: log-linear plot, showing exponential distribution.

E. Plot of distribution of instantaneous intraburst spike frequencies (from 372 intraburst ISIs). Parameters: mean spike rate 5.21 Hz, coefficient of variation (CV) 1.29, fraction of spikes in bursts 0.48, mean burst rate 0.64 Hz, interburst CV 1.38, mean spikes per burst 3.9, mean burst duration 130 ms, burst duration CV 0.49, mean intraburst ISI 44.5 ms, mean instantaneous intraburst spike rate 25.01 Hz, intraburst CV 0.29.

F–H. Mitral cell with non-bursting spontaneous spike activity.

F. Segment of a cell-attached voltage clamp recording of action currents (length 20 s).

G. Linear-log plot of interspike interval (ISI) distribution for entire record (356 s, 3368 spikes) showing unimodal distribution. Inset: log-linear plot showing nearly exponential distribution.

H. Plot of joint ISI distribution of all spikes. Parameters: mean spike rate 4.03 Hz, CV 0.90.

Figure 3. Distributions of spike train parameters of mitral and tufted cells

Comparison of distributions of parameters characterizing spiking and bursting patterns of tufted cells (upper panels) and mitral cells (lower panels). Data is from 83 tufted cells (34 non-bursting, 49 bursting) and 41 mitral cells (21 non-bursting, 20 bursting).

A. Mean spike rate (number of spikes/duration of recording), non-bursting cells.

B. Mean spike rate, bursting cells.

C. Coefficient of variation (CV), non-bursting cells.

D. CV, bursting cells.

E. Burst threshold frequency.

F. Burst fraction (fraction of spikes in bursts).

G. Mean burst rate (number of bursts/duration of recording).

H. Interburst CV (standard deviation of interburst interval/mean interburst interval).

I. Mean number of spikes per burst.

J. Mean burst duration.

K. Mean intraburst spike rate.

L. Intraburst CV.

Figure 4. Correlated firing in tufted-tufted cell pairs

A. Fluorescence image of olfactory bulb slice with a number of tufted cells loaded by electroporation with tetramethylrhodamine dextran. The somata of two recorded tufted cells are indicated by arrows. Magnified inset: overlapped fluorescent primary dendritic tufts of recorded cells. Scale bar: 100 μm . Inset scale bar: 15 μm .

B. Pair of tufted cells that were filled with biocytin after whole-cell recording, showing convergent primary dendrites (p) and overlapping apical tufts (t). Laterally projecting secondary dendrites (s) are also stained in both cells. Scale bar: 100 μm . Right panel: Sketch of recording configuration from tufted-tufted cell pair with convergent dendrites.

Abbreviations. EPL, external plexiform layer; GL, glomerular layer; MCL, mitral cell layer.

C. Current traces from cell-attached recording of spontaneous spike activity in a pair of tufted cells with slow correlations in firing.

D. Poisson-normalized spike cross-correlogram (10 ms bin) for tufted-tufted cell pair in **C** (dark trace), showing a single broad peak (225 s record, 863 spikes from upper cell, 654 from lower cell).

E. Voltage traces from whole-cell recording of spontaneous spike activity in a pair of tufted cells with both slow and fast correlations in firing.

F. Poisson-normalized spike cross-correlogram (10 ms bin) for tufted-tufted cell pair in **E** (dark trace), showing superposed broad and narrow peaks with comparable heights (223 s record, 327 spikes from upper cell, 272 from lower cell).

G. Current traces from cell-attached recording of spontaneous spike activity in a pair of tufted cells with strong fast correlation in firing.

H. Poisson-normalized spike cross-correlogram (10 ms bin) for tufted-tufted cell pair in **G** (dark trace), showing narrow peak (338 s record, 1491 spikes from upper cell, 1657 from lower cell). Light gray traces in **D**, **F** and **H** are cross-correlograms for time-shuffled data

Figure 5. Properties of correlated firing in tufted-tufted cell pairs

A. Poisson-normalized spike cross-correlogram (10 ms bin) for a tufted-tufted cell pair (dark trace) with a large narrow peak and small broad peak (304 s record, 2153 and 3104 spikes). The broad peak was fitted with a Lorentzian function (smooth curve, $H = 1.44 \pm 0.13$, $W = 0.59 \pm 0.05$ s, $L = 0.056 \pm 0.014$ s). Light gray trace is cross-correlogram for time-shuffled data.

B. Expanded plot showing fine structure of large central peak of cross-correlogram (1 ms bin) of cell pair in **A**, with broad peak fit function subtracted. The narrow peak resolved into two moderately narrow sub-peaks split symmetrically about the center, and a sharp center peak occupying a single 1 ms bin. The two split peaks were fitted with a double Lorentzian curve (smooth curve, $H_1 = 6.97 \pm 0.171$, $W_1 = 4.1 \pm 0.8$ ms, $L_1 = -3.7 \pm 0.2$ ms, and $H_2 = 6.88 \pm 1.35$, $W_2 = 6.9 \pm 1.1$ ms, $L_2 = 5.6 \pm 0.3$ ms; fit with center bin removed).

C. Plot of central peak of cross-correlogram (1 ms bin) of another cell pair, with only a narrow peak present (no broad peak). The peak resolved into a moderately narrow peak and a sharp center peak of width < 2 ms. Smooth curve: Lorentzian fit to moderately narrow peak ($H = 8.70 \pm 2.30$, $W_1 = 19.3 \pm 3.5$ ms, $L_1 = 7.1 \pm 1.3$ ms; fitted with 2 ms peak removed).

D. Log-log plot of magnitude of peak lag (L) versus peak width (W) for 34 significant cross-correlogram peaks (including sub-peaks) from tufted-tufted cell pairs. For sharp peaks in a single 1 ms bin, we took $W = 1$ ms. Upper inset: linear-log histogram showing bimodal peak width distribution with gap (log W bin width = 0.4). Right inset: sketch showing Lorentzian parameters for fitting cross-correlograms: height (H), width (W) and lag (L) (see Methods).

E. Histogram comparing mean center heights (ch), a measure of fast correlation, of tufted-tufted pairs with divergent (0.15 ± 0.04 , $n = 8$) and convergent (1.93 ± 0.49 , $n = 14$) primary dendrites with narrow peaks (values are mean $\pm$ SEM).

F. Histogram comparing mean side areas (sa), a measure of slow correlation, of tufted-tufted pairs with divergent (-0.0025 ± 0.01093 , $n = 8$) and convergent (0.265 ± 0.101 , $n = 13$) primary dendrites with broad peaks (values are mean $\pm$ SEM). Upper right inset: sketch of center height (ch) and side area (sa) parameters describing normalized cross-correlograms.

G. Scatter plot of heights (H) of narrow peaks in cross-correlograms ($W < 50$ ms) against side area (sa), showing independence of fast and slow correlation of tufted-tufted pairs (gray line is least squares fit).

H. Scatter plot of interspike interval coefficient of variation (CV) for tufted cell pairs with convergent dendrites, showing correlation in firing pattern ($R = 0.64$, $P = 0.0042$, $n = 18$ pairs, bursting and non-bursting).

I. Scatter plot of fraction of spikes in bursts for tufted cell pairs with convergent dendrites, showing correlation in degree of bursting ($R = 0.87$, $P = 0.001$, $n = 10$ bursting pairs).

J. Scatter plot of mean burst rate for tufted cell pairs with convergent dendrites, showing correlation in frequency of bursting ($R = 0.63$, $P = 0.049$, $n = 10$ bursting pairs). Gray lines in G–J are least squares fits.

Figure 6. Correlated firing in mitral-tufted cell pairs

A. Fluorescence image of a mitral-tufted cell pair with convergent primary dendrites (tufted cell on left, mitral cell on right). Alexa Fluor 594 was dialyzed into both cells during whole-cell recording. Scale bar: 100 μ m. Right panel: sketch of recording configuration from mitral-tufted cell pair with convergent dendrites. Abbreviations: see Fig. 4B..

B. Current traces from cell-attached recording of spontaneous spike activity in a mitral-tufted cell pair with slow correlations in firing.

C. Poisson-normalized spike cross-correlogram (10 ms bin) for mitral-tufted cell pair in **B** (dark trace), showing a single broad peak (270 s record, 357 spikes from upper cell, 3131 from lower cell).

D. Current traces from cell-attached recording of spontaneous spike activity in a mitral-tufted cell pair with both slow and fast correlations in firing.

E. Poisson-normalized spike cross-correlogram (10 ms bin) for mitral-tufted cell pair in **D** (dark trace), showing superposed broad and narrow peaks with comparable heights (452 s record, 1090 spikes from upper cell, 1683 from lower cell).

F. Current traces from cell-attached recording of spontaneous spike activity in a mitral-tufted cell pair with strong fast correlation in firing.

G. Poisson-normalized spike cross-correlogram (10 ms bin) for mitral-tufted cell pair in **F** (dark trace), showing narrow peak (450 s record, 467 spikes from upper cell, 656 from lower cell). Light gray traces in **C**, **E** and **G** are cross-correlograms for time-shuffled data

Figure 7. Properties of correlated firing in mitral-tufted cell pairs

A. Poisson-normalized spike cross-correlogram (10 ms bin) for a mitral-tufted cell pair (dark trace) with a large narrow peak (450 s record, 1746 mitral cell spikes, 1214 tufted cell spikes). Light gray trace is cross-correlogram for time-shuffled data.

B. Expanded view (1 ms bin) of fine structure of central peak of the cross-correlogram of cell pair in **A**. The narrow peak resolved into two moderately narrow sub-peaks split symmetrically about the center. The two peaks were fitted with a double Lorentzian curve (smooth curve, $H_1 = 2.98 \pm 0.61$, $W_1 = 8.9 \pm 1.5$ ms, $L_1 = -7.8 \pm 0.4$ ms, and $H_2 = 1.93 \pm 0.74$, $W_2 = 5.9 \pm 1.8$ ms, $L_2 = 8.4 \pm 0.5$ ms).

C. Log-log plot of magnitude of peak lag (L) versus peak width (W) for 34 significant cross-correlogram peaks (including sub-peaks) from mitral-tufted cell pairs. For sharp peaks in a single 1 ms bin, we took $W = 1$ ms. Inset: linear-log histogram showing bimodal peak width distribution (log W bin width = 0.4).

D. Ordered plot of lags of narrow peaks in the cross-correlogram for mitral-tufted cell pairs. Negative lag corresponds to mitral cell spike preceding tufted cell spike.

E. Histogram comparing mean side areas (sa), a measure of slow correlation of mitral-tufted pairs with divergent (0.0025 ± 0.0098 , $n = 7$) and convergent (0.221 ± 0.077 , $n = 18$) primary dendrites with broad peaks (values are mean $\pm$ SEM).

F. Histogram comparing mean center heights (ch), a measure of fast correlation of mitral-tufted pairs with divergent (0.050 ± 0.025 , $n = 7$) and convergent (1.70 ± 1.09 , $n = 13$) primary dendrites with narrow peaks (values are mean $\pm$ SEM).

G. Scatter plot of the heights (H) of narrow peaks ($W < 50$ ms) against side area (sa), showing independence of fast and slow correlations for mitral-tufted pairs (gray line is least squares fit).

H. Scatter plot of interspike interval coefficient of variation (CV) for mitral-tufted cell pairs with convergent dendrites, showing correlation in firing pattern ($R = 0.49$, $P = 0.015$, $n = 24$ pairs). Gray lines in **G** and **H** are least squares fits.

Figure 8. Enhancement of tufted cell burst firing by NBQX

A–E. Tufted cell spontaneous spike activity in control condition.

A. Segment of cell-attached voltage clamp recording of action currents (38 s) with detected burst spikes indicated above in a raster plot of spike times.

B. Linear-log plot of interspike interval (ISI) distribution for entire record (304 s, 2153 spikes) showing multimodal distribution. Inset: log-linear plot showing non-exponential distribution. Arrow: bursting threshold (20 ms).

C. Plot of joint ISI distribution of all spikes. Gray lines: 20 ms ISI threshold; open circles: burst spikes; filled circles: non-burst spikes.

D. Plot of the distribution of interburst intervals (IBI) (from 139 bursts). Inset: log-linear plot, showing exponential distribution.

E. Plot of distribution of instantaneous intraburst spike frequencies (from 425 intraburst ISIs) *Parameters:* mean spike rate 7.08 Hz, coefficient of variation (CV) 2.40, fraction of spikes in bursts 0.27, mean burst rate 0.48 Hz, interburst CV 1.59, mean spikes per burst 3.9, mean burst duration 35.72 ms, burst duration CV 1.01, mean intraburst ISI 12.32 ms, mean instantaneous intraburst spike rate 109.11 Hz, intraburst CV 0.22.

F–J. Spontaneous spike activity recorded from the tufted cell in **A–E**, after perfusion with 20 μ M NBQX.

F. Segment of action current recording (38 s) with detected burst spikes indicated as raster plot, showing change to more strongly bursting pattern of firing.

G. Linear-log and log-linear (inset) histograms of interspike interval (ISI) distribution for entire record in NBQX (190 s, 1133 spikes) showing bimodal distribution Arrow: altered bursting threshold (85 ms).

H. Plot of joint ISI distribution of all spikes in NBQX. Gray lines: 85 ms ISI threshold; open circles: burst spikes; filled circles: non-burst spikes.

I. Plot of the distribution of interburst intervals (IBI) (from 53 bursts), showing increased number of longer IBIs. Inset: log-linear plot, showing exponential distribution of short IBIs.

J. Plot of distribution of instantaneous intraburst spike frequencies (from 1021 intraburst ISIs) *Parameters:* mean spike rate 6.00 Hz, coefficient of variation (CV) 5.26, fraction of spikes in bursts 0.96, mean burst rate 0.31 Hz, interburst CV 1.03, mean spikes per burst 18.67, mean burst duration 596 ms, burst duration CV 0.86, mean intraburst ISI 33.8 ms, mean instantaneous intraburst spike rate 39.23 Hz, intraburst CV 0.51.

Figure 9. Variation in the effect of NBQX on tufted cell firing

A–C. Tufted cell with non-bursting spontaneous spike activity in control condition.

A. Segment of a cell-attached voltage clamp recording of action currents (length 20 s).

B. Linear-log and log-linear histograms of interspike interval (ISI) distribution for entire record (400 s, 4554 spikes) showing unimodal distribution.

C. Plot of joint ISI distribution of all spikes. *Parameters:* mean spike rate 11.37 Hz, CV 1.03.

D–F. Spontaneous spike activity recorded from the tufted cell in **A–C**, after perfusion with 20 μ M NBQX.

D. Segment of cell-attached voltage clamp recording of action currents (length 20 s).

E. Linear-log and log-linear (inset) histograms of interspike interval (ISI) distribution for entire record in NBQX (600 s, 7627 spikes) showing unimodal distribution.

F. Plot of joint ISI distribution of all spikes in NBQX. *Parameters:* mean spike rate 12.70 Hz, CV 1.80.

G–K. Normalized ratio plots showing changes of spike and burst parameters for records in NBQX (filled circles, $n = 20$ cells, $n = 13$ bursters) and records in control condition for other cells without NBQX (open circles, $n = 8$ cells, $n = 7$ bursters) obtained over similar perfusion times (> 400 s): mean spikes rate (**G**), ISI coefficient of variation (CV) (**H**), mean burst rate (**I**), fraction of spikes in bursts (**J**), and mean burst duration (**K**). Asterisk in **J** and **K** marks the same cell as shown in Figure 8.

Figure 10. Effect of NBQX on mitral cell firing

A–E. Mitral cell spontaneous spike activity in control condition.

A. Segment of cell-attached recording of action currents (60 s) with detected burst spikes indicated above in a raster plot of spike times.

B. Linear-log and log-linear (inset) histograms of ISI distribution for entire record (400 s, 1343 spikes) showing bimodal distribution. Arrow: bursting threshold (110 ms).

C. Plot of joint ISI distribution of all spikes. Gray lines: 110 ms ISI threshold; open circles: burst spikes; filled circles: non-burst spikes.

D. Histogram of distribution of interburst intervals (IBI) (152 bursts). Inset: log-linear plot.

E. Histogram of instantaneous intraburst spike frequencies (839 intraburst ISIs).

Parameters: mean spike rate 3.36 Hz, coefficient of variation (CV) 3.38, fraction of spikes in bursts 0.74, mean burst rate 0.39 Hz, interburst CV 1.39, mean spikes per burst 6.45,

mean burst duration 324 ms, burst duration CV 0.665, mean intraburst ISI 59.5 ms, mean instantaneous intraburst spike rate 19.1 Hz, intraburst CV 0.36.

F–J. Spontaneous spike activity recorded from the mitral cell in **A–E**, after perfusion with 20 μ M NBQX, showing no significant change from control.

F. Segment of cell-attached recording of action currents (60 s) with detected burst spikes indicated as raster plot.

G. Linear-log and log-linear (inset) histograms of interspike interval (ISI) distribution for entire record in NBQX (300 s, 904 spikes) Arrow: invariant bursting threshold (110 ms).

H. Plot of joint ISI distribution of all spikes in NBQX. Gray lines: 110 ms ISI threshold; open circles: burst spikes; filled circles: non-burst spikes.

I. Histogram of interburst intervals (IBI) (79 bursts). Inset: log-linear histogram.

J. Histogram of instantaneous intraburst spike frequencies (1021 intraburst ISIs).

Parameters: mean spike rate 3.00 Hz, coefficient of variation (CV) 4.58, fraction of spikes in bursts 0.78, mean burst rate 0.27 Hz, interburst CV 1.35, mean spikes per burst 8.68, mean burst duration 463 ms, burst duration CV 0.88, mean intraburst ISI 60.2 ms, mean instantaneous intraburst spike rate 18.87 Hz, intraburst CV 0.34.

K–O. Normalized ratio plots showing changes of spike and burst parameters for records in NBQX (filled circles, $n = 12$ cells, $n = 7$ bursters) and records for other cells in control condition without NBQX (open circles, offset right, $n = 10$ cells, $n = 3$ bursters) obtained over similar perfusion times (> 400 s): mean spikes rate (**K**), ISI coefficient of variation (CV) (**L**), mean burst rate (**M**), fraction of spikes in bursts (**N**), and mean burst duration (**O**).

Figure 11. Effect of NBQX on correlated firing in tufted-tufted and mitral-tufted cell pairs

A. Narrow center peaks of Poisson-normalized cross-correlogram (1 ms bin) of a tufted-tufted cell pair before (left panel) and after (right panel) perfusion of the slice with 20 μ M NBQX. Double Lorentzian peak fit parameters (smooth curves) were: control: $H_1 = 6.97 \pm 1.71$, $W_1 = 4.1 \pm 0.78$ ms, $L_1 = -3.67 \pm 0.24$ ms, and $H_2 = 6.88 \pm 1.35$, $W_2 = 6.93 \pm 1.07$ ms, $L_2 = 5.61 \pm 0.33$ ms; NBQX: $H_1 = 9.27 \pm 2.12$, $W_1 = 4.20 \pm 0.78$ ms, $L_1 = -6.2 \pm 0.26$ ms, and $H_2 = 8.40 \pm 2.97$, $W_2 = 2.49 \pm 0.73$ ms, $L_2 = 3.5 \pm 0.21$ ms.

B. Normalized ratio plots showing changes in narrow peak height (left panel) and narrow peak width (right panel) in cross-correlograms of tufted-tufted cell pairs with convergent primary dendrites (filled circles: NBQX, 12 peaks; open circles: control, 3 peaks).

C. Broad center peaks of Poisson-normalized cross-correlogram (10 ms bin) of a tufted-tufted cell pair before (left panel) and after (right panel) perfusion of the slice with 20 μ M NBQX. Lorentzian peak fit parameters (smooth curves) were: control: $H = 4.00 \pm 0.28$, $W = 0.5 \pm 0.03$ s, $L = -0.14 \pm 0.01$ s; NBQX: $H = 5.92 \pm 0.312$, $W = 0.50 \pm 0.02$ s, $L = 0.14 \pm 0.008$ s.

D. Normalized ratio plots showing changes in broad peak height (left panel) and broad peak width (right panel) in cross-correlograms of tufted-tufted cell pairs with convergent primary dendrites (filled circles: NBQX, 6 peaks; open circles: control, 2 peaks).

E. Narrow center peaks of Poisson-normalized cross-correlogram (1 ms bin) of a mitral-tufted cell pair before (left panel) and after (right panel) perfusion of the slice with 20 μ M NBQX. Lorentzian peak fit parameters (smooth curves) were: control: $H = 23.84 \pm 2.16$, $W = 16.8 \pm 1.2$ ms, $L = -2.15 \pm 0.4$ ms; NBQX: $H = 26.33 \pm 3.26$, $W = 45.4 \pm 4.6$ ms, $L = -2.04 \pm 1.5$ ms.

F. Normalized ratio plots showing changes in narrow peak height (left panel) and narrow peak width (right panel) in cross-correlograms of mitral-tufted cell pairs with convergent primary dendrites (filled circles: NBQX, 6 peaks; open circles: control, 4 peaks).

G. Broad center peaks of Poisson-normalized cross-correlogram (10 ms bin) of a mitral-tufted cell pair before (left panel) and after (right panel) perfusion of the slice with 20 μ M NBQX. Lorentzian peak fit parameters (smooth curves) were: control: $H = 1.76 \pm 0.06$, $W = 1.56 \pm 0.04$ s, $L = 0.015 \pm 0.012$ s; NBQX: $H = 3.19 \pm 0.163$, $W = 0.77 \pm 0.03$ s, $L = -0.106 \pm 0.011$ s.

H. Normalized ratio plots showing changes in broad peak height (left panel) and broad peak width (right panel) in cross-correlograms of mitral-tufted cell pairs with convergent primary dendrites (filled circles: NBQX, 5 peaks; open circles: control, 5 peaks).

In **B**, **D**, **F** and **H**, changes in NBQX (filled circles) are compared to spontaneous changes in control condition for other pairs without NBQX (open circles) obtained over similar perfusion times (> 400 s).

Figure 12. Electrical coupling of tufted cells

A. Electrical coupling in a tufted-tufted cell pair with convergent primary dendrites, measured during double whole-cell recording of membrane currents under voltage-clamp. Tufted cell #1 was hyperpolarized from -60 mV to -200 mV (upper trace, cell input conductance 4.43 nS), and an outward current recorded in tufted cell #2 clamped at -60 mV (lower trace). This yielded a reverse voltage coupling coefficient of $C_{21} = \Delta V_1 / \Delta V_2 = \Delta I_2 / \Delta I_1 = 0.086$.

B. Measurement of forward voltage coupling coefficient for the tufted-tufted pair in **A**, by hyperpolarizing tufted cell #2 from -60 mV to -200 mV (lower trace, cell input conductance 5.34 nS), and measuring outward current in tufted cell #1 (upper trace), $C_{12} = 0.067$.

C. Electrical coupling in a mitral-tufted cell pair with convergent primary dendrites, measured during double whole-cell recording of membrane voltages under current-clamp. Mitral cell was hyperpolarized by -500 pA current injection (upper trace, cell input conductance 9.29 nS), and a hyperpolarization was recorded the tufted cell (lower trace). This yielded a forward voltage coupling coefficient of $C_{12} = \Delta V_2 / \Delta V_1 = 0.017$.

D. Measurement of reverse voltage coupling coefficient for the mitral-tufted pair in **C**, by hyperpolarizing -500 pA current injection into tufted cell (lower trace, cell input conductance 3.43 nS), and measuring hyperpolarizing voltage deflection in mitral cell (upper trace), $C_{21} = 0.0106$.

Figure 13. Electrically coupled networks and cross-correlograms

Diagrams of different excitation pathways in electrically coupled networks of mitral or tufted cells that could account for a variety of observed narrow peak structures in spike cross-correlograms. Each cell in a coupled chain is assumed to initiate a spike upon electrical excitation by the previous cell in the chain..

A. A single moderately narrow cross-correlogram peak with zero lag can be generated by symmetric excitation of two recorded cells by an upstream cell. The peak is broadened by spike timing variability of polysynaptic pathways.

B. A single moderately narrow cross-correlogram peak with non-zero lag can be generated by polysynaptic excitation of one recorded cell by the other. Spike timing variability of the

polysynaptic pathway broadens the peak, and the delay of the pathway shifts it off-center. One-way propagation might result from differences in impedances of connected cells that block reversed spike initiation across one or more gap junction couplings.

C. A cross-correlogram with twin moderately narrow peaks with positive and negative lags can be generated by mutual polysynaptic excitation of two recorded cells. The peak is broadened by spike timing variability of polysynaptic pathways. Forward and reverse excitation may propagate through a two-way pathway (left diagram), or two separate one-way pathways (right diagram).

D. A cross-correlogram with sharp center peak and twin moderately narrow peaks with positive and negative lags can be generated by adding to the circuits in **C** either a direct electrical coupling between the two recorded cells (left diagram) or direct electrical excitation of the two cells by strong voltage input from an upstream cell (right diagram).

Comparison of the variation of spiking and bursting parameters of spontaneously firing mitral and tufted cells in olfactory bulb slices. Number of cells in each sample is indicated in parentheses. See Methods for definitions of parameters.

Table 1

	Mitral cells			Tufted cells		
	range	mean $\pm$ SD	median	range	mean $\pm$ SD	median
mean spike rate (Hz) (non-bursting cells)	0.45 – 9.48	4.18 $\pm$ 3.07 (21)	3.13	0.68 – 20.15	5.60 $\pm$ 5.15 (34)	3.34
mean spike rate (Hz) (bursting cells)	0.90 – 17.80	6.14 $\pm$ 4.62 (20)	4.60	0.40 – 17.22	5.99 $\pm$ 3.78 (49)	5.32
CV (non-bursting cells)	0.08 – 3.06	1.45 $\pm$ 0.86 (21)	0.94	0.55 – 3.84	1.39 $\pm$ 0.71 (34)	1.18
CV (bursting cells)	0.64 – 7.96	2.45 $\pm$ 1.62 (20)	1.94	0.35 – 4.28	1.05 $\pm$ 0.61 (49)	0.98
burst threshold (bursting cells) (Hz)	4.0 – 33.3	12.47 $\pm$ 6.47 (20)	12.13	5.26 – 50	15.9 $\pm$ 12.3 (49)	10.75
burst fraction	0.15 – 0.98	0.62 $\pm$ 0.30 (20)	0.70	0.07 – 1.00	0.63 $\pm$ 0.29 (49)	0.72
mean burst rate (Hz)	0.087 – 1.99	0.60 $\pm$ 0.57 (20)	0.37	0.02 – 2.18	0.58 $\pm$ 0.49 (49)	0.42
interburst CV	0.472 – 1.56	1.09 $\pm$ 0.39 (20)	1.12	0.35 – 4.28	1.05 $\pm$ 0.61 (49)	0.98
mean spikes per burst	3.53 – 45.33	8.03 $\pm$ 9.11 (20)	5.77	3.08 – 47.7	8.02 $\pm$ 8.05 (49)	5.49
mean burst duration (s)	0.08 – 1.06	0.30 $\pm$ 0.23 (20)	0.25	0.020 – 1.51	0.26 $\pm$ 0.24 (49)	0.22
burst duration CV	0.40 – 1.13	0.61 $\pm$ 0.18 (20)	0.60	0.28 – 1.01	0.59 $\pm$ 0.16 (49)	0.55
mean intraburst ISI (ms)	10.3 – 89.3	50.8 $\pm$ 18.8 (20)	46.4	8.8 – 89.2	42.75 $\pm$ 23.0 (49)	42.7
mean intraburst spike rate (Hz)	12.1 – 98.9	29.5 $\pm$ 18.3 (20)	25.51	13 – 164	42.9 $\pm$ 33.6 (49)	28.34
intraburst CV	0.17 – 0.65	0.35 $\pm$ 0.13 (20)	0.33	0.17 – 0.69	0.38 $\pm$ 0.10 (49)	0.38