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Vikrant Kapoor
Carnegie Mellon University

Nathaniel N. Urban
Carnegie Mellon University, nurban@cmu.edu

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Glomerulus-Specific, Long-Latency Activity in the Olfactory Bulb Granule Cell Network

Vikrant Kapoor and Nathaniel N. Urban
Department of Biological Sciences and Center for the Neural Basis of Cognition, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213

Reliable, stimulus-specific temporal patterns of action potentials have been proposed to encode information in many brain areas, perhaps most notably in the olfactory system. Analysis of such temporal coding has focused almost exclusively on excitatory neurons. Thus, the role of networks of inhibitory interneurons in establishing and maintaining this reliability is unclear. Here we use imaging of population activity in vitro to investigate the mechanisms of temporal pattern generation in mouse olfactory bulb inhibitory interneurons. We show that activity of these interneurons evolves slowly in time but that individual neurons fire at reliable times, with a timescale similar to the slow changes in the patterns of odor-evoked activity and to odor discrimination. Most strikingly, the latency of a single granule cell is highly reliable from trial to trial during repeated stimulation of the same glomerulus, whereas this same cell will have a markedly different latency when a different glomerulus is activated. These data suggest that the timing of granule cell-mediated inhibition in the olfactory bulb is tightly regulated by the source of input and that inhibition may contribute to the generation of reliable temporal patterns of mitral cell activity.

Key words: olfactory bulb; mitral cell; granule cell; glomerulus; calcium imaging; activation latency

Introduction

The olfactory bulb (and related structures in invertebrates) performs many functions that are remarkably simple, although it is a relatively simple neuronal network (Schoppa and Urban, 2003). The architecture of the olfactory bulb is characterized by reciprocal dendrodendritic synapses between predominantly two cell types: principal cells, mitral/tufted cells, and the local interneurons, granule cells (Rall et al., 1966; Price and Powell, 1970; Jahr and Nicoll, 1980; Shepherd and Greer, 1998). These circuits mediate inhibitory modulation of mitral cell activity (Laurent, 1999; Laurent et al., 2001) and are believed to be critical for refinement of initial odor-evoked mitral cell activity (Yokoi et al., 1995; Friedrich and Laurent, 2001; Luo and Katz, 2001; Shimshek et al., 2005).

Action potential timing is thought to play a key role in coding and computation in the olfactory system. Olfactory stimuli result in reproducible patterns of action potentials lasting tens or hundreds of milliseconds after odor onset (Hamilton and Kauer, 1989; Stopfer and Laurent, 1999; Margrie et al., 2001). Generating such patterns requires reliably timed inhibition, although relatively little is known about the timing of activity of inhibitory interneurons.

Recent work has focused considerable attention on the behavioral and physiological analyses of events that occur between 200 and 800 ms after the onset of odor-evoked activity (Friedrich and Laurent, 2001; Uchida and Mainen, 2003; Abraham et al., 2004; Friedrich et al., 2004). Patterns of odor-evoked activity that are initially highly correlated become progressively more decorrelated over the first several hundred milliseconds after odor stimulation (Friedrich and Laurent, 2001; Stopfer et al., 2003). This decorrelation enhances the discriminability of patterns of mitral cell activity. Evolution of these activity patterns occurs over a time period of hundreds of milliseconds (~250–800 ms), during which spiking patterns of principle cells (mitral cells and projection neurons) are odor-specific and remarkably reliable trial to trial (Laurent et al., 1996; Wehr and Laurent, 1999; Margrie et al., 2001). Behavioral experiments also have shown that a similar timescale (up to ~600 ms) is required for rodents to make difficult odor discriminations (Abraham et al., 2004; Rinberg et al., 2006) (also see Uchida and Mainen, 2003). Both time-dependent decorrelation and behavioral discrimination are likely to involve lateral inhibitory interactions between principle cells (Yokoi et al., 1995; MacLeod and Laurent, 1996; Stopfer et al., 1997), most probably mediated by granule cells (Shimshek et al., 2005). Interestingly, lateral inhibition between mitral cells has been shown to have slow rise and decay times (Urban and Sakmann, 2002), reaching peak amplitude in ~100 ms and having a decay time of ~350 ms.

The evolution and reliability of such temporal spiking patterns will depend on the inhibitory circuitry of the olfactory bulb. Here we investigate the properties of and mechanisms underlying the slow evolution of dendrodendritic inhibition, focusing on the activity of granule cells. By monitoring the activity of populations...
of granule cells in vitro, we find that granule cells fire with long but reliable latencies after stimulation of single mitral cells or populations of mitral cells. Although latencies ranged widely across cells, latencies of single granule cells were input specific, thus linking the timing of inhibitory modulation of mitral cell activity to odor identity.

Materials and Methods

Slice preparation
Slices were prepared using methods adapted from previous work (Urban, 2002). Mice (2–3 weeks old, C57BL/6) were deeply anesthetized with a mixture of 0.1% ketamine and 0.1% xylazine (~100 µl dose, monitored by responsiveness to tail pinch) and then decapitated. Brains were removed and placed in ice-cold Ringer’s solution (in mM: 125 NaCl, 25 glucose, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 1 MgCl₂, and 2.5 CaCl₂). Sagittal or coronal slices (300 µm thick) of olfactory bulbs were cut using a vibratome (VT1000S; Leica, Nussloch, Germany). After cutting, slices were incubated in the above Ringer’s solution (continuously oxygenated) at 37°C for 30 min before being loaded with calcium dye.

Electrophysiology
Whole-cell recordings were made using patch pipettes filled with internal buffer (130 mM potassium gluconate, 10 mM HEPES, 2 mM MgCl₂, 2 mM MgATP, 2 mM Na₂ATP, 0.3 mM GTP, 4 mM NaCl, and, in some cases, 1% bicytisin), using a Multiclamp 700A amplifier (Molecular Devices, Palo Alto, CA) and IT&C-18 data acquisition board (Instrutech, Port Washington, NY). Cells were visualized under infrared differential interference contrast (DIC) optics (BX51WI; Olympus Optical, Tokyo, Japan). Physiological data were collected via software written in Igor Pro (WaveMetrics, Lake Oswego, OR). Depending on the type of cells, appropriate whole-cell patch pipettes (1–3 MΩ for mitral cells and 5–10 MΩ for granule cells) were used to make current-clamp recordings. Extracellular stimulation was delivered via theta glass electrodes pulled to a tip size of ~1 µm using a single constant-current pulse (300 µs). All of the experiments were done at 35°C in modified Ringer’s solution (in mM: 125 NaCl, 25 glucose, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 0.2 MgCl₂, 2.5 CaCl₂) unless stated otherwise.

Fura-2/fura-4 FM loading
Olfactory bulb slices were transferred to a special chamber containing 500 µl of Ringer’s solution in which they were loaded with membrane-permeable calcium indicators using methods adapted from previous work in cortical slices. Humidified air was passed above the surface of liquid in the chamber to keep the solution oxygenated. To the incubation chamber, we added 3 µl of 0.01% pluronic (Invitrogen, Carlsbad, CA) and 5 µl of a 1 mM solution of either fura-2 or fura-4 FM (Invitrogen) in 100% DMSO solution Slices were incubated in this solution at 37°C for 60–90 min. Data reported here were collected with fura-2-loaded slices unless stated otherwise. After tissue loading, the tissue is much more fluorescent than control tissue, suggesting that considerable dye is contained in neuropil structures that are not individually identifiable (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). However, under our conditions, the neuropil staining does not show stimulus-evoked changes in fluorescence, as indicated in supplemental Figure 3 (available at www.jneurosci.org as supplemental material).

Imaging
After loading, slices were placed in a submersion recording chamber under an upright microscope using 20, 40, or 60× water immersion objectives (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). For a particular experiment, an objective was chosen to give the best tradeoff between field of view (330 vs 165 vs 110 µm) and brightness of the image (numerical aperture of 0.5, 0.8 or 0.9). Slices were visualized for successful loading using excitation wavelength of 360–400 nm and emission wavelength of 480–520 nm. Experiments were performed at 37°C unless stated otherwise. A cooled back-illuminated frame transfer CCD camera (Micromax 512 BFT; Princeton Instruments, Trenton, NJ) was used to capture single fluorescent images and sequences of images (movies). Exposure times were 30–60 ms per frame with 3 × 3 binning (final image size, 170 × 170 pixels). Images were acquired and stored using software written in Igor Pro (WaveMetrics) using drivers (SIDX) from Bruxton Scientific (Seattle, WA).

After loading, extracellular stimulation elicited decreases in fluorescence in many imaged neurons. In response to a given stimulus, only a fraction of the cells visibly labeled in the raw fluorescence image showed calcium transients. To investigate the response profile of granule cells, we acquired 2-s-long movies (30–60 ms/frame) of the granule cell layer in fura-2-loaded slices after glomerular stimulation (300 µs constant-current pulse). Movies were analyzed by calculating ΔF/F-frame by frame for whole movies and then identifying cells showing decreases of fluorescence across the frames of individual movies. The response profiles of different granule cells were plotted as ΔF/F (change in fluorescence/basal fluorescence) versus time by calculating the change in average pixel values over a particular region of interest. We chose not to perform background subtraction before calculating ΔF/F because identifying a background region in which fura fluorescence did not contribute to the total signal was not possible. This presumably reflects the fact that calcium dye is distributed in the cytoplasm of cell bodies and neuronal processes throughout the thickness of the slice.

Data analysis
The response profiles of different granule cells were plotted as ΔF/F (change in fluorescence/basal fluorescence) versus time by calculating the change in average pixel values over a particular region of interest. These regions of interest were the size of single granule cells and, in many cases, were identifiable as single cells in either fluorescence or the DIC image. Activation latency was calculated as the time of first deviation from the basal fluorescence, and rise time was the difference between the time of first deviation and time taken to attain the peak fluorescence. We used the total rise time of granule cell calcium transients as a measure of total duration of granule cell activity.

Probability. Probability of a single cell was defined as the ratio of number of trials on which a given cell showed any measurable activity to the total number of trials (experiments in which a minimum of 10 trials was performed were used to calculate this probability). The probability index was calculated as mean of probability of all of the cells imaged for a given condition.

For IPSC reliability analysis, we used custom mini-analysis software to detect IPSCs and then convolved these train of events with a square function of width 50 ms. These convolved trials were used to calculate the pairwise cross-correlation across trials.

The correlogram computed from these data were compared with the same computed from simulated trials. To generate simulated IPSC trains, we computed the average rate of events for all trials (50 trials). New simulated spike trains were generated randomly from this measured time-dependent frequency of events.

Analysis. For clustering trials based on response latency vectors or binary activity vectors, we calculated the distance between a vector representing a given trial and a vector that was the ideal vector (defined below) for all of the trials on which a given glomerulus was stimulated. Specifically, dis = ||vector|Gi| × |vector|gj| – |vector|gj| × |vector|Gi|, where |vector|Gi is ideal response vector for ith glomerulus (i = 1 or 2), |vector|gj is trial vector for jth trial (j = 1–20, 10 trials each for two glomeruli). For clustering analysis based on latencies, |vector|Gi is the vector of mean response latencies for granule cells (for all trials on which the cell was activated). For clustering analysis based on binary activity, |vector|Gi is a vector giving the reliabilities of granule cell activity in response to stimulation of a given glomerulus. After computing the distance a trial was from the two ideal vectors, we plotted each trial in two dimensions to generate Figure 7, G and H.

To calculate the correlation matrix in Figure 8, the activation probability of a single cell was calculated as the proportion of the total trial activity shown by that cell. Pairwise coactivation probability (data) was
Figure 1. Kinetics of recurrent and lateral IPSPs. A, Mitral cell voltage trace in response to current injection (300 pA for 100 ms) showing a series of spikes, followed by long-lasting recurrent inhibition. B, Same mitral cell voltage response to current injection after the addition of 10 μM gabazine to the bath. C, Enlarged view of mitral cell voltage trace showing the presence of many high-frequency inhibitory synaptic events. D, E, Mitral cell (MT) voltage traces for two mitral cells (bottom trace is an average), in response to current injection (400 pA for 100 ms) in cell 1, showing the time course of inhibition (lateral) in the second cell. F, G, Hypotheses to explain the duration of recurrent and lateral IPSPs. F, Hypothesis A: single granule cells are persistently active. After a single stimulus, individual granule cells maintain activity with a time course comparable with that of recurrent (or lateral) inhibition. G, Hypothesis B: granule cell latencies are widely distributed. Different granule cells have different activation latencies that are randomly distributed along the time course of dendrodendritic (recurrent/lateral) inhibition.

Figure 2. Response latencies and durations of granule cell activity for glomerular stimulation. A, Experimental setup for recording granule cell calcium transients in response to glomerular stimulation. B, C, Raw fluorescence and glomerular stimulation-evoked activity in the granule cells, respectively. D, Granule cell calcium transients for cells shown in C in response to glomerular stimulation. The arrow depicts the time of stimulation. E, Granule cell activation latency histogram for glomerular stimulation (average latency, 368 ± 18 ms; n = 256), showing widely distributed activation latencies. F, Granule cell rise time histograms for glomerular stimulation (average, 448 ± 14 ms), showing widespread distribution of granule cell rise times.
ular layer ranged widely, from 0 to 900 ms, with a mean of
granule cell calcium transients after stimulation of the glomer-

shown in Figure 2,

available at www.jneurosci.org as supplemental material). As
granule cell calcium transients after stimulation of the glom-
ular layer ranged widely, from 0 to 900 ms, with a mean of
368 ± 18 ms (n = 256 granule cells). We also quantified the
rise times of granule cell calcium transients after glomerular
stimulation to get an indication of the duration of granule cell
spiking. The rise times of granule cell calcium transients were
also widely distributed (mean of 448 ± 14 ms; n = 256) (Fig.
2 F). These results demonstrate asynchronous and long-
latency recruitment of granule cells after activation of popula-
tions of mitral cells. Additionally, activity of individual gran-
ule cells persists for hundreds of milliseconds after their initial
activation, as indicated by the long rise times of the calcium
transients. We did not analyze the spatial patterns of granule
cell activity, in part because of concerns that these patterns
would be influenced by slice angle and other biologically un-
interesting details of the experiment. However, we typically
found the maximum density of activated granule cells in the
superficial granule cell layer directed beneath the stimulated
glomerulus.

We investigated possible mechanisms underlying the unusu-
ally slow patterns of granule cell activity elicited by glomerular
stimulation. We first asked whether our stimulation resulted
in asynchronous activation of mitral cells, which then gener-
ated asynchronous granule cell activity. To test this hypothe-
sis, we examined the distribution of mitral cell activation
latencies in response to glomerular stimulation (Fig. 3A–C).
We found that the majority (~98%; n = 38) of the mitral
cells showed calcium transients that were visible in the first
frame recorded after the stimulus (30–60 ms frame rate)
(Fig. 3 B, C). Thus, the wide distribution of granule cell activa-
tion latencies is not a result of long latencies of mitral cell
firing.

Next, we examined the granule cell latencies in response to
direct stimulation of the granule cell layer to determine whether
the kinetics of granule cell activity were attributable to intrinsic
excitability of granule cells or whether they were a specific con-
sequence of activation of granule cells by mitral cells (Fig. 3E–G).
Extracellular stimulation in granule cell layer, which should
result in direct activation of granule cells and also activation by
ascending axons, caused granule cell activity with short latencies
and rise times (100% of these cells were active in the first frame
after the stimulation, with average rise times of 15 ± 17 ms; n =
57) (Fig. 3 E, F). This indicates that the long-latency granule cell
responses observed above are not attributable to intrinsic kinetics
of granule cell firing or to our method of measuring activation
time and duration, but rather are a feature of granule cell activa-
tion by mitral cells.

Given the large ranges in the latency to granule cell activity
and duration of this activity, we wondered whether short-latency
events also had long rise times. Such a relationship would suggest
that the long-latency events occur preferentially in weakly ac-
tivated granule cells, which should also produce only a few
action potentials and thus short rise times. In fact, we found
no relationship between response latency and response rise
time (Fig. 3D) (r = 0.13). This suggests that the long latency
and the long duration of the granule cell responses are gener-
ated by different mechanisms and indicates that the long-
latency responses are unlikely to be caused simply by low levels
of input. We considered examining the relationship between
latency and amplitude of calcium transients, but cell-to-cell
differences in dye concentration in bulk-loaded tissue would
influence the amplitude of calcium transients, making inter-
pretation of amplitude data suspect.
Figure 4. Response latencies and duration of granule cell activity for single mitral cell stimulation. A, Experimental setup for imaging granule cell activity in response to single mitral cell stimulation. B, C, Raw fluorescence and ΔF/Δt image showing single mitral cell stimulation-evoked activity, respectively. D, Granule cell calcium transients for cells shown in C. The arrow depicts the time of stimulation. E, Granule cell activation latency histogram for glomerular stimulation (average latency, 357 ± 34 ms; n = 78), showing widely distributed activation latencies. F, Plot of granule cell response latencies versus granule cell rise time for glomerular stimulation. There was no correlation between granule cells activation latencies and granule cells rise time (r = 0.22).

Figure 5. Blockade of transient potassium channels alter granule cell latencies. A, Granule cell activation latency distributions: red, normal conditions (average latency, 368 ± 18 ms; n = 256); blue, 2.5 mM 4-AP (average, 310 ± 17 ms; n = 242); brown, 5.0 mM 4-AP (average, 213 ± 20 ms; n = 108); green, 10.0 mM 4-AP (average, 182 ± 16 ms; n = 149), showing shift in granule cell activation latency distribution after blocking A-current. Curves have been normalized to the peak of the curves. B, Granule cell activation latency distributions for conditions described in A. Curves have been normalized to the area under curves. C, Plot for average rise times and average latencies under different concentrations of 4-AP, showing no significant difference in the total rise time of granule cell calcium transients under different conditions (mean rise time was 448 ± 14 ms for normal condition, 423 ± 15 ms for 2.5 mM 4-AP, 452 ± 22 ms for 5 mM 4-AP, and 456 ± 19 ms for 10 mM 4-AP) (p > 0.1 for Wilcoxon’s rank sum test). D, Experimental setup for measuring decay time constant of granule cell-mediated inhibition. E1, E2, 4-AP resulted in faster granule cell-mediated inhibition and reduced the time-to-peak of inhibition. The arrow depicts the stimulation time. E3, 4-AP reduced the decay time constant of granule cell-mediated inhibition. OX-314, 2-(Triethylamino)-N-(2,6-dimethylphenyl) acetamine.

Asynchrony of granule cell activation can be reduced by blockade of A-type potassium channels

We next investigated how this asynchronous activity is generated across a population of granule cells and whether the differences in latencies across granule cells are attributable to different intrinsic properties of the granule cells. Previous work (Schoppa and Westbrook, 1999) has shown that granule cells express a high density of A-type potassium channels, which are believed to be important for generating delayed firing. Thus, it is possible that different granule cells express different amounts of A-current and thus have different latencies. If this is true, then blockade of A-current should reduce long-latency events and increase the degree to which granule cells fire synchronously. Thus, we used fura imaging to record granule cell activity after glomerular stimulation under different concentrations of the A-current blocker 4-aminopyridine (4-AP). Blockade of A-current resulted in a marked shift in the distribution of granule cell latencies (Fig. 5A–C). The mean response latency shifted from 368 ± 18 ms (normal conditions; n = 256) to 182 ± 16 ms for 10 mM 4-AP (n = 182; p < 0.01 for Wilcoxon’s rank sum test). [Mean response latencies were 310 ± 17 ms for 2.5 mM 4-AP (n = 242) and 213 ± 20 ms for 5 mM 4-AP (n = 109).] Because granule cell latencies were more homogeneous when A-current was blocked, this suggests that the slow time course of granule cell-mediated inhibition...
Granule cells are activated with low probability but with reliable timing

In initial experiments, we observed that granule cells were activated at relatively low probability across trials. To determine the probability with which granule cells were activated, we imaged the activity of a population of granule cells in response to glomerular stimulation for large numbers of trials (≥10 with intertrial interval of approximately 20 s). As shown in Figure 6, A1 and A2, granule cells were activated with relatively low probability (granule cells responded in 15 ± 4% of the total recorded trials; n = 46). Despite this low probability of firing, when granule cells responded, the amplitude and latency of their calcium transients were remarkably reliable across trials (Fig. 6A2). The mean SD for granule cell latencies across trials was 60.6 ms (Fig. 6C) (n = 24). We further investigated whether blocking A-current altered the probability of granule cell activity. Application of 10 mM 4-AP resulted in a marked increase in the probability of granule cell firing across trials (30 ± 4% for 2.5 mM 4-AP, 49 ± 5% for 5 mM 4-AP, and 58 ± 6% for 10 mM 4-AP; n = 79) (Fig. 6B1–B3). Application of 4-AP did not seem to affect the trial-to-trial reliability of activation latencies (Fig. 6B2).

Granule cell-mediated IPSCs show reliable latencies

The observation that granule cells fire with latencies that are reliable to within 60 ms suggests that granule cell-mediated inhibition also should be temporally reliable at a similar timescale. To investigate the reliable timing of inhibition observed in mitral cells, we recorded IPSCs from mitral cells (voltage clamped at −40 mV) in response to repeated stimulation (50 sweeps) of a single glomerulus (Fig. 6D). To determine whether some IPSCs occurred at consistent latencies, we first identified the time of IPSC peaks and then convolved these trains of events with a square function of width 50 ms. This convolution means that the data on IPSC timing were effectively binned with 50 ms bins, allowing us to use the same sampling rate for both imaging and patch-clamp data. We then calculated the pairwise correlation coefficients for these binned data for the group of 50 trials. These sweeps showed a relatively high average correlation coefficient (0.65 ± 0.002) (Fig. 6E). To determine whether the computed cross-correlation is different from that observed by chance, we compared this value with the same value computed from simulated event trains having the same bin-by-bin time-dependent frequency of events (for details, see Materials and Methods) (Fig. 6E). We observed significantly higher correlation for IPSCs recorded from the mitral cells than for the simulated events (0.63 ± 0.02 for actual events vs 0.55 ± 0.02 for simulated events; n = 5 cells; p < 0.01 for Kolmogorov–Smirnov test) (Fig. 6F). This indicates that some IPSCs occur reliably (to within 50 ms) at fixed times after the stimulation across many trials, consistent with our data from imaging granule cell activity. Higher precision reliability was not evaluated in these experiments.

Granule cell latencies are reliable and glomerulus specific

Odor-evoked mitral cell firing often consists of periods of increases and decreases in firing rate that occur over hundreds of milliseconds. In many cases, these complex patterns are odor specific and are reliably evoked across repeated presentations of the same odor (Friedrich and Laurent, 2001). Blockade of inhibition alters the patterns of odor-evoked firing in mitral cells (Yokoi et al., 1995; Margrie et al., 2001) and in their insect analogs (Stopfer et al., 1997), and thus it seems likely that dendrodendritic inhibition plays a role in generating these odor-specific firing patterns. We tested whether the latency and/ or kinetics of granule cell activity in our experiments is reliable across repeated stimulation of the same glomerulus and whether these features of the response of single granule cells are different when a single granule cell is activated by stimulation of different glomeruli.

We examined the latencies to initial activity across populations of granule cells after extracellular stimulation in neighboring pairs of glomeruli (Fig. 7A). When neighboring glomeruli were stimulated, we saw 15–30% overlap in the populations of active granule cells. For this subset of granule cells, we then compared the latencies of calcium transients after stimulation of the different glomeruli and also after repeated stimulation in the same glomerulus (Fig. 7F). Latencies of single granule cells differed when two different glomeruli were stimulated (Fig. 7F, yellow circles) (Wilcoxon’s rank sum test; p ≤ 0.01), whereas, consistent with the data above, latencies for single granule cells were very similar when a single glomerulus was stimulated repeatedly (p > 0.5 for Wilcoxon’s rank sum test) (Fig. 7F, red and green triangles). We calculated the difference in latencies for granule cells in response to glomerular stimulation for two consecutive trials and in response to stimulation of two different
glomeruli, and these differed significantly. This finding shows that granule cell activation latencies are glomerulus specific, which in turn suggests that granule cell latencies may effectively code which glomeruli are active. To test whether latency data were sufficient to determine which glomerulus was stimulated, we represented each trial as a vector (of length 12), in which each element represented the latency of one granule cell (20 trials, 10 each for two different glomeruli; inter-trial interval was \( \frac{450}{20} \) s). We then projected each trial vector on to ideal response vectors \([\text{vector}]_{G1L} \) and \([\text{vector}]_{G2L}\); see Materials and Methods) for both glomeruli. This projection analysis was used as a way of depicting the similarity of trials in the plane. We observed a clear glomerulus-specific clustering of trials and distinct separation of the trials depending on which glomerulus was stimulated (Fig. 7G). Hence, the latencies of granule cell activity provide sufficient information to identify the activated glomerulus. We repeated the same analysis to determine whether the identity of activated granule cells (within the set of cells that could be activated by both glomeruli) could be used to determine which glomerulus was stimulated. In this case, trial vectors represented only the activity state of granule cells on a given trial (1 if granule cell was active and 0 if granule cell was inactive). Each of these trials was then projected on to ideal response vectors \([\text{vector}]_{G1R} \) and \([\text{vector}]_{G2R}\); see Materials and Methods). These projections were not clustered, and, thus, within the subset of cells activated by either glomerulus, the identity of the activated cells on a given trial did not provide information sufficient to identify the active glomerulus (Fig. 7H).

**Granule cell activity is uncorrelated across the population and across trials**

We next investigated the degree to which activity of granule cells was correlated both within and across trials. Specifically, we wanted to determine whether subsets of granule cells were coactive more often than predicted by their individual response probabilities (within trial correlation) and whether the probability of granule cell firing varied in a consistent manner from trial to trial (across trial correlation). We recorded the activity of populations of granule cells (Fig. 8A1, A2) in response to glomerular stimulation for at least 10 trials (intertrial intervals of \( \sim \frac{40}{20} \) s). We first calculated the probability of firing for each cell across all the trials and
we used these probabilities to predict the joint probability that these cells would be coactive (assuming that their activities were independent of each other). These results were displayed as a matrix in which the value at each point (row, column) shows the probability that the cells in the given row and column are both activated (Fig. 8, data in B1 and predicted coactivation probabilities in B2). Thus, the diagonal elements give the probability of firing for each cell. If the activity of these granule cells is pairwise independent, then the predicted value of the off-diagonal elements can be determined by multiplying the probabilities of activity for the cells in the given row and column. In Figure 8, as summarized in B3 and evident from B1 and B2, actual and predicted coactivation probabilities were highly correlated ($r = 0.8$ for $n = 76$ cells). This implies that the coactivation observed is consistent with the assumption that individual granule cells are activated independently. The only potential deviation from independence was found in looking at activity of the cells with the lowest probability of being activated. In this case, the probability of rarely active cells being coactive was somewhat higher than predicted by chance.

Discussion

Dendrodendritic inhibition in the mammalian olfactory bulb is critical for regulating both the frequency and timing of mitral cell spiking, yet many features of this phenomenon and the mechanisms that generate them are not well understood. Here we analyzed the activity of ensembles of granule cells after mitral cell activity using population imaging after bulk loading of calcium indicator. We show that granule cell population activity consists of spiking at widely ranging latencies (0–1000 ms), that these latencies are reliable across trials, and that the latencies are specific to stimulation of particular glomeruli. The duration of granule cells activity was independent of granule cell activation latencies, suggesting that longer latencies cannot be explained simply as being attributable to less input to the granule cells. These data indicate that glutamate release from mitral cells results in asynchronous recruitment of large populations of granule cells having reliable patterns of latencies, consistent with the idea that these patterns may play a role in shaping the slowly evolving temporal patterns of mitral cell activity.

One of the most remarkable aspects of our findings is that the latency of granule cell activity is reliable and specific to stimulation of a particular glomerulus. That is, glomerular identity is coded by the latency of granule cell firing. In an in vivo context, this observation would lead us to predict that granule cell latencies would be odor specific and thus that mitral cells might show reliable pauses in firing time locked to stimulus onset when stimulated with specific odorants. Such odor-specific patterns of mitral cell activity have in fact been observed and may be an important part of odor coding (Hamilton and Kauer, 1989; Wehr and Laurent, 1996; Luo and Katz, 2001; Margrie et al., 2001; Wilson et al., 2004). Although various circuit-level explanations have been proposed for such observations, our data suggest that the interaction of mitral and granule cells alone may be sufficient to explain these phenomena. Such slow changes in mitral cell activity also may result in time-dependent decorrelation of mitral cell activity (Friedrich and Laurent, 2001). This decorrelation of initially similar odor-evoked activity patterns into functionally distinct patterns develops over a timescale of hundreds of milliseconds (~400 ms). Such time-dependent decorrelation re-
quires consistent modulation of odor-evoked activity over prolonged periods after odor onset, such as could be achieved via the glomerulus-specific long-latency responses we report here.

Although such slow changes at the physiological level have not been directly tied to behavior, odor discrimination in rodents has been shown to occur during a period of between 200 and 550 ms after odor onset (Uchida and Mainen, 2003; Abraham et al., 2004), and some studies have found that the time depends on the difficulty of the task performed (Abraham et al., 2004; Rinberg et al., 2006). However, this result is somewhat controversial (Friedrich, 2006; Uchida et al., 2006). Our results show that brief mitral cell activity results in spiking in granule cells over this entire period. This delayed spiking suggests that, for several hundred milliseconds after mitral cell activity, the firing of granule cells will be sensitive to additional activity in mitral cells and also in their other inputs to granule cells (e.g., projections from cortical areas). That is, such long-latency activity gives the granule cell network a prolonged time window over which to integrate additional activity. The duration of this window may be sufficient to allow integration of information across successive sniff cycles and may be important for reliable relay of information to downstream neurons.

Our results also raise the question of the mechanism by which these reliable long-latency responses are generated. In general, long-latency responses can be generated by slowly activating inward currents, such as persistent sodium currents or by slowly inactivating outward currents, such as A-type potassium currents. Inactivation of potassium channels generates long delays to spiking in many systems, and we have shown that blockade of A-current results in an ~50% reduction in the latency of granule cell activity. Blockade of A-current also may affect other olfactory bulb neurons, but the concentration dependency of the effect that we observe suggests that it is mediated by Kv4.X potassium channels, which are expressed predominantly in granule cells. We do see that 4-AP makes mitral cells more likely to fire repeatedly after stimulation, but this would not explain the decreased latency of granule cell spiking. However, even after blockade of A-current, latencies are rather long (180 ms average) and rather widely distributed, suggesting that other mechanisms also contribute to long-latency spiking. Moreover, the inactivation time constant of A-current in granule cells has been measured to be in the tens of milliseconds (Schoppa and Westbrook, 1999), indicating that, although A-current is important for delaying spiking, the timing of the delay is not directly attributable to the inactivation but rather involves other mechanisms as well (Molineux et al., 2005). Similarly, the observation that a single granule cell may show very different latencies to stimulation of different glomeruli indicates that, if intrinsic properties determine the latency, they must interact differently with different inputs. An intriguing possibility is that input to different dendrites results in different latencies to spiking. If firing latencies are specific to the site at which synapses are made onto granule cell dendrites, then the role of A-current in prolonging spiking latencies could be attributable to differences in A-channel density on different dendritic branches.

Other candidate mechanisms include the combination of NMDA receptors (Schoppa et al., 1998; Chen et al., 2000; Habibsky et al., 2000) and voltage-gated calcium channels (Isaacson, 2001), both of which can generate relatively long-lasting responses under appropriate conditions. In our experiments, we use a low concentration of Mg$^{2+}$ that will favor the activation of NMDA receptors. However, we have also seen long-latency granule cell activity in solutions containing 1.0 mM Mg$^{2+}$ (our unpublished observations). Lowering Mg$^{2+}$ increases substantially the number of active cells that we observe in response to single mitral cell or single glomerulus stimulation. Granule cells have been shown to fire low-threshold calcium spikes (Egger et al., 2003, 2005; Pinato and Midtgaard, 2003, 2005) that sometimes generate depolarizations in these cells lasting tens of hundreds of milliseconds. Even longer depolarizations have been observed in granule cells in amphibia, which are attributable in part to calcium-activated nonspecific cationic currents (Hall and Delaney, 2002). In most other systems, long latencies to spiking are associated with a high degree of temporal jitter, but this is not the case in our experiments on granule cell activity.
Indeed, granule cell show remarkable reliability in their response latencies, even when the probability of firing is rather low. The fact that latencies are glomerulus specific indicates that the biophysical mechanisms that give rise to the long latencies must be in some respect input specific. This may be attributable to different inputs activating different dendritic branches of the granule cell (Zelles et al., 2006) or otherwise to different properties of synapses onto the granule cells. The biophysical mechanisms underlying this unique behavior are unclear, but understanding them will be important as we try to understand the functional role of granule cells in olfactory bulb circuitry.

A prediction of our data is that these long-latency granule cell spikes will alter the firing pattern of mitral cells, which will in turn alter granule cell latencies. That is, delayed inhibition by granule cells will eventually shape the activity of other granule cells. This dynamic feedback will be especially important in the case of prolonged mitral cell activity, including odor-evoked activity. Our observation that activity of different granule cells is only weakly correlated in single sweeps suggests that such dynamic feedback may be critical for generating coincident activity in populations of granule cells, as reported by Schoppa (2006) and, as has previously suggested, may be critical for synchronizing mitral cell firing (Lagier et al., 2004; Galan et al., 2006). Additional work on the interplay of ongoing granule and mitral cell activity will be needed to understand how these processes shape patterns of ongoing mitral cell activity.

References


